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ALLOPATRY AND ANATOMICAL DISTINCTIVENESS OF TWO PUZZLING LAND SNAILS IN GENUS *PLAGIODONTES*, FROM ARGENTINA (GASTROPODA: ORTHALICIDAE, ODONTOSTOMINAE)

Julia Pizá* & Néstor J. Cazzaniga

Universidad Nacional del Sur, Departamento de Biología, Bioquímica y Farmacia,
San Juan 670, (8000) Bahía Blanca, Argentina

ABSTRACT

Plagiodontes daedaleus (Deshayes, 1851) is the most widespread species in the genus *Plagiodontes* Doering, 1877. Although some 19th century authors recorded some differences in shell shape between the populations living to the east or to the west of the Pampean Sierras, Argentina, such differences were ignored by later authors. A multivariate statistical analysis on 608 adult shells collected at 29 localities in and around these mountains was used to test if such narratively described shell differences were statistically significant, irrespectively of their taxonomic status. Shells classified as collected from the east or the west of the sierras were correctly identified in 91.9% of the cases by Multivariate Discriminant Analysis, leading to recognizition of two allopatric shell morphologies. These external divergences were found to correspond with anatomical differences in the reproductive system. We conclude that two different, allopatric species were merged under the name *Plagiodontes daedaleus* in the literature and raise the subspecies *Plagiodontes daedaleus strobelii* (Doering, 1877) to the specific level. Another subspecies living to west of the sierras, *Plagiodontes daedaleus salinicola* (Doering, 1877), also showed a simple penis papilla and a non-swollen vagina, which shows that *salinicola* is not a member of *P. daedaleus* but rather a population of *P. strobelii* that does not deserve subspecific status. The taxonomic significance of the presence of either a simple penial papilla or a papilla bearing an accessory lobe was also confirmed for these *Plagiodontes* species.

Key words: shell morphometry, pallial complex, reproductive system, *Plagiodontes strobelii*, taxonomy, Stylommatophora.

INTRODUCTION

Plagiodontes daedaleus (Deshayes, 1851) is known to be the most variable, widespread species in genus *Plagiodontes* Doering, 1877 (Parodiz, 1939; Fernández, 1973). Although some 19th century authors (Strobel, 1874; Doering, 1877, 1878) recorded noteworthy differences in shell shape between the populations found to the east or to west of the Pampean Sierras, Argentina, such differences were not taken into account by later authors. So, the literature merged under a single specific name many shells of variable obesity, the size of which ranges from medium to large, and which have either a blunt spire or a comparatively acute one.

After intensive sampling in and around these sierras, together with a detailed examination of samples stored in the principal museums of Argentina, we have found that those long-observed, subjectively detected shell differences were demonstrable by multivariate statistic analysis, and that they concurred with anatomical differences in the reproductive system that warrant recognition as two allopatric species.

The species living to the west of the sierras had already received the subspecific name *P. daedaleus major* (Strobel, 1874), which was replaced by *P. daedaleus strobelii* (Doering, 1877) to avoid homonymy with *P. daedaleus major* (Pfeiffer, 1853). The confusion of these two forms created long-lasting taxonomic confusion.

The aims of this paper are to test shell differences between populations of "*Plagiodontes daedaleus*" living over opposite slopes of the Pampean Sierras and to considerably enlarge

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*Corresponding author: jpiza@uns.edu.ar

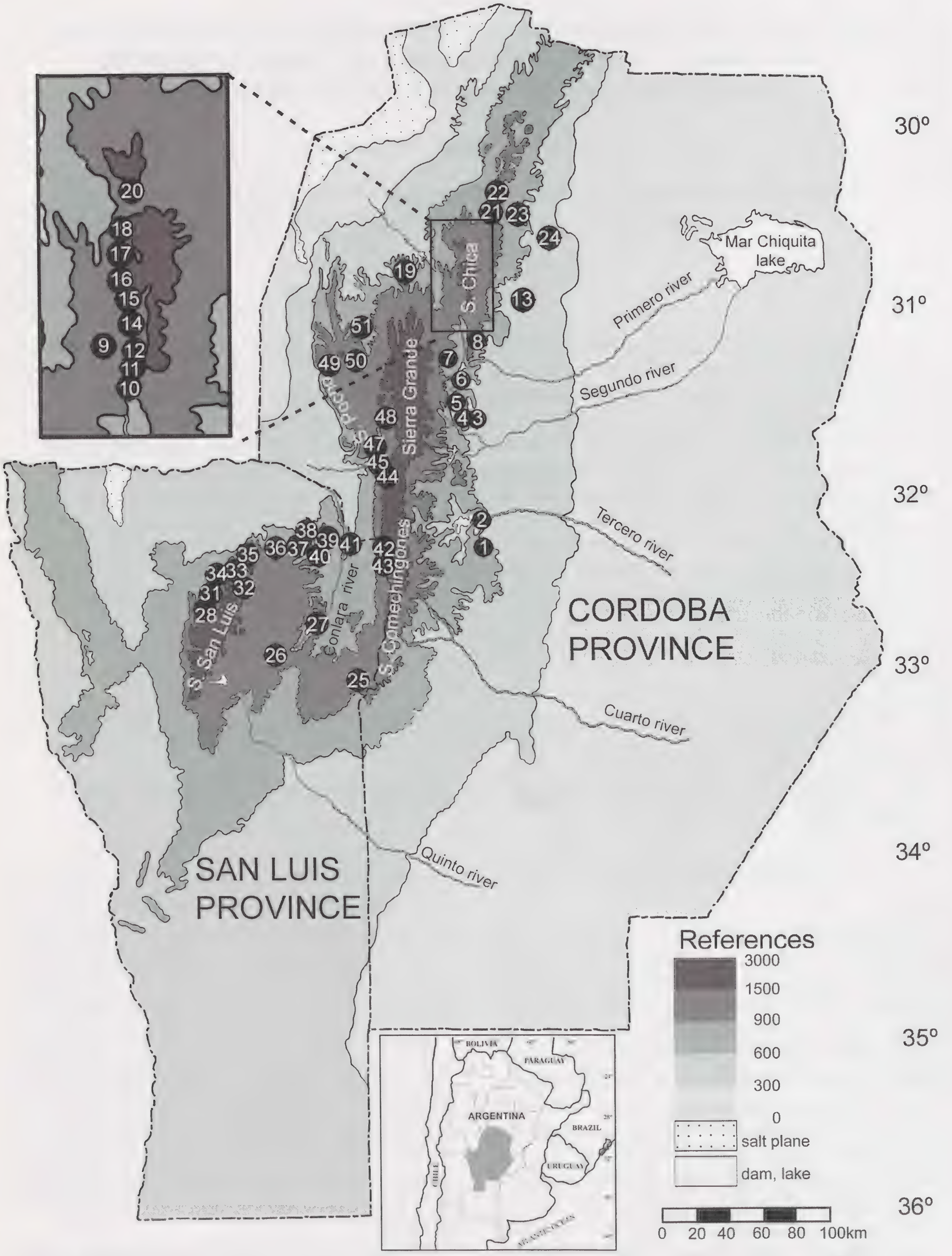


FIG. 1. Study area in the Pampean Sierras, Córdoba and San Luis provinces, Argentina. Localities indicated according to Appendix 1.

the description of the internal anatomy of the studied taxa, paying particular attention to the inner structure of penis and vagina, which are studied here for the first time.

According to our results, *Plagiodontes strobelii* (Doering, 1877) is raised to the species level and its distribution is described as allopatric with *P. daedaleus* (Deshayes, 1851).

MATERIAL AND METHODS

Study Area

The sampling area covered part of the Pampean Sierras and their surrounding areas in the provinces of Córdoba and San Luis, central Argentina (Fig. 1). They form a mountain complex 400 km long, of N-S direction from 33°30'S to 29°20'S and from 64°30'W to 66°30'W. This chain consists of parallel mountain ranges, the dominant of which is the central Sierra Grande and Sierra de Comechingones, highest summits of which are Champaquí (2,884 m) and Los Gigantes (2,880 m), respectively. The system of the Pampean Sierras constitutes an important geographic barrier that delimits a clear east-west climate differentiation.

The general climate is mountainous subhumid, with a marked temperature gradient due to altitude. Rainfall increases on the eastern slopes owing to the cooling and condensation of warm, humid winds from the Atlantic Ocean.

The studied area covers part of the phytogeographical provinces of Chaco and Espinal (Cabrera & Willink, 1973). Highest mountains are mainly characterized by mountain forest ("bosque serrano"), which gradually becomes a xerophytic forest in the piedmont; both kinds of woods are similar, but plant heights and species diversity are lower in the Espinal than in the Chaco province. The summits of the sierras are covered by mountain grasslands.

Allopatry and Shell Variability

Intensive fieldwork was carried out during January 2005, on the forests on both sides of Sierra Grande, Sierra de Comechingones and Sierra de San Luis. The material collected in the field was complemented by samples from the collections of Museo Argentino de Ciencias Naturales (MACN), Buenos Aires, Museo de La Plata (MLP), La Plata, and Fundación Miguel Lillo (FML), San Miguel de Tucumán (Appendix 1).

Shell variability was analyzed on 608 adult specimens from 29 localities. According to external features, most shells were identifiable either as *Plagiodontes daedaleus daedaleus* or *P. daedaleus strobelii,* though the identity of a few specimens was doubtful. Adulthood was assumed for shells that showed full development of the apertural folds and lamellae, together with a reflected outer aperture lip. Seven linear variables were measured on shell drawings made with the aid of a camera lucida device fitted to a stereoscopic microscope: shell length (SL), shell width (SW), length of the last whorl (LWL), width of the last whorl (LWW), aperture length (AL), major apertural length (MAL) and aperture width (AW); major and spiral angles were also determined as described in Pizá & Cazzaniga (2003).

Multivariate Principal Component Analysis (PCA) and Multivariate Discriminant Analysis (MDA) were applied to quantitative data using the SPSS statistical package.

Descriptors of measurements and proportions (mean, standard deviation, coefficient of variation and range) were calculated. The significance of differences in linear measurements and their proportions between groups was checked by t-tests using the SPSS statistical package.

Shell Morphology

Characteristics of apertural folds and lamellae and number of whorls were determined through direct observation under a stereoscopic microscope. Protoconch and teleoconch sculpture of juvenile and adult shells was analyzed with a JEOL 35 CF scanning electronic microscope.

Internal Anatomy

Pallial and genital systems were studied on 15 specimens of each form (*P. daedaleus daedaleus* and *P. daedaleus strobelii*), collected at the localities indicated in Appendix 1.

Living specimens were drowned in cold, previously boiled water, fixed and conserved in 70% ethanol. Dissections and illustrations were made with the aid of a Zeiss Stemi SV6 stereoscopic microscope fitted with a camera lucida device.

Terminology used to describe the genital system follows Tompa (1984) and Gómez (2001).

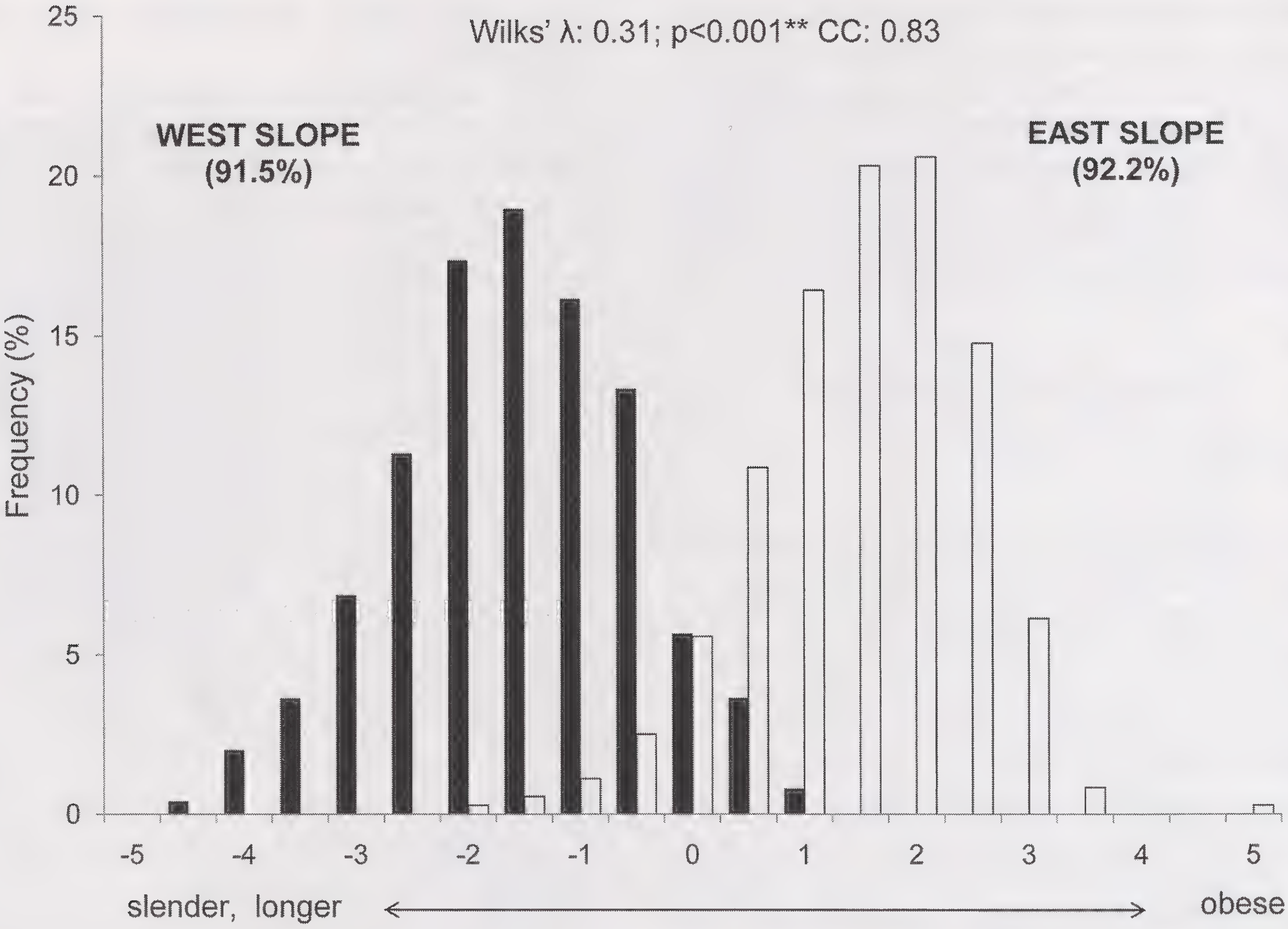


FIG. 2. Frequency histogram of the scores of discriminant function 1 of the MDA performed on shell measurements of *Plagiodontes* from the eastern and western slopes of the Pampean Sierras in Córdoba and San Luis provinces. Correct classification percentages, Wilks' λ, and canonical correlation (CC) are indicated.

RESULTS

Allopatry of Shell Morphologies

MDA on 608 shells classified according to their origin (west or east of the sierras) resulted in the following classification functions:

$Y_{\text{west slope}}$ = -7.84 SL + 66.04 SW - 21.52 LWL + 30.49 LWW + 31.64 AL - 15.98 AW - 43.12 AML + 20.23 MA + 3.16 SA - 1464.70

$Y_{\text{east slope}}$ = -8.29 SL + 65.52 SW - 24.48 LWL + 33.05 LWW + 34.12 AL - 13.16 AW - 44.66 AML + 20.16 MA + 3.26 SA - 1458.41

Cross validation results of this analysis (91.9% correct classifications) support the hypothesis that these two morphs are allopatric (Table 1, Fig. 2).

Shell Comparison of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*

Some representative specimens of the two taxa are shown in Figures 3 and 4. Morphometric information obtained from the studied shells is summarized in Table 2; means of all variables differed significantly (p << 0.001).

PCA of shell parameters revealed two Principal Components accounting for 85.9% of the total variance (Table 1). The scatter plot in Figure 5 shows that the specimens of *P. daedaleus strobelii* had predominantly negative PC2 scores. This is mainly due to its more slender shell, as evidenced by higher values of major angle and lower values of spiral angle. Conversely, the scores of P. *daedaleus daedaleus* form a cloud in the positive range of PC2 due to their obese shell.

The position of the samples on the PC1-PC2 plane was fully consistent with their geographical distribution.

Shell Morphology (Table 3)

Protoconch and Teleoconch Sculpture: The protoconch of *P. daedaleus daedaleus* has 2–2.25 whorls, with undulated axial striae and some anastomoses in the first half whorl (Fig. 6); it has straight ribs decussated by spiral lines in the two last protoconch whorls (Fig. 7). The limit between protoconch and teleoconch is well defined (Fig. 8). The teleoconch has a variable striation. Some

TABLE 1. Summary of the multivariate analyses performed on shell measurements of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*. MDA-Geo: Structure matrix of the MDA performed on *Plagiodontes* shells classified according to their origin from the east (N = 360) or west (N = 248) slope of the Pampean Sierras (Córdoba and San Luis provinces, Argentina). ACP-Taxa: Component matrix of the PCA performed on 608 shells of *Plagiodontes strobelii* and *P. daedaleus*. Abbreviations: AL, aperture length; AML, aperture major length; AW, aperture width; LWL, last whorl length; LWW, last whorl width; MA, major angle; NW, number of whorls; SA, spiral angle; SL, shell length; SW, shell width.

	MDA-Geo Structure Matrix	ACP-Taxa Component Matrix	
Variable	DF1	PC 1	PC 2
SL	-0.48	0.77	-0.57
SW	0.11	0.82	0.43
LWL	-0.31	0.91	-0.33
LWW	0.23	0.74	0.56
AL	-0.10	0.96	-0.04
AW	0.21	0.76	0.38
MAL	-0.09	0.96	0.00
MA	-0.57	0.08	-0.90
SA	0.63	-0.23	0.85

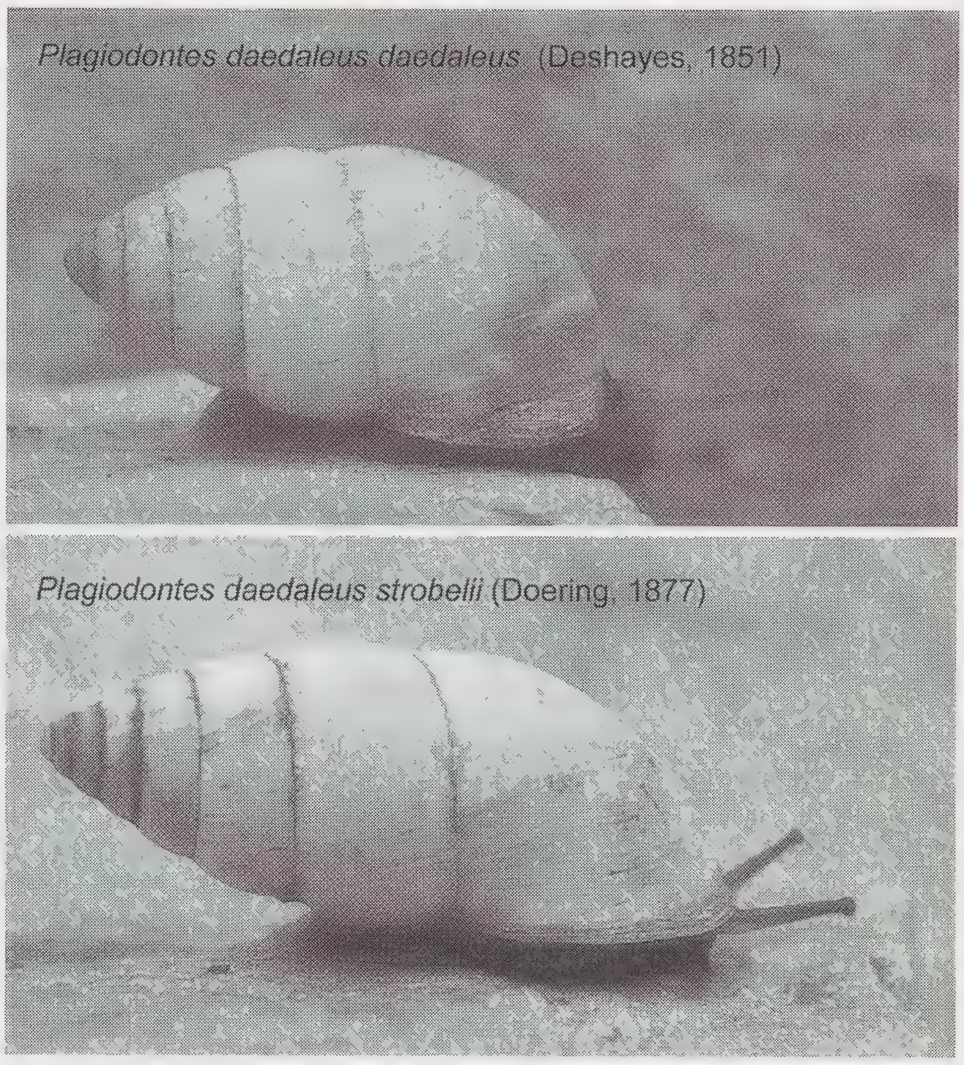

Plagiodontes daedaleus daedaleus (Deshayes, 1851)
Plagiodontes daedaleus strobelii (Doering, 1877)

FIG. 3. Living specimens of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*.

shells that look almost smooth have a slight sculpture, which is visible at low magnification under a stereomicroscope. The shells usually have axial striae in the first post-nepionic whorls (Fig. 9), which are gradually replaced

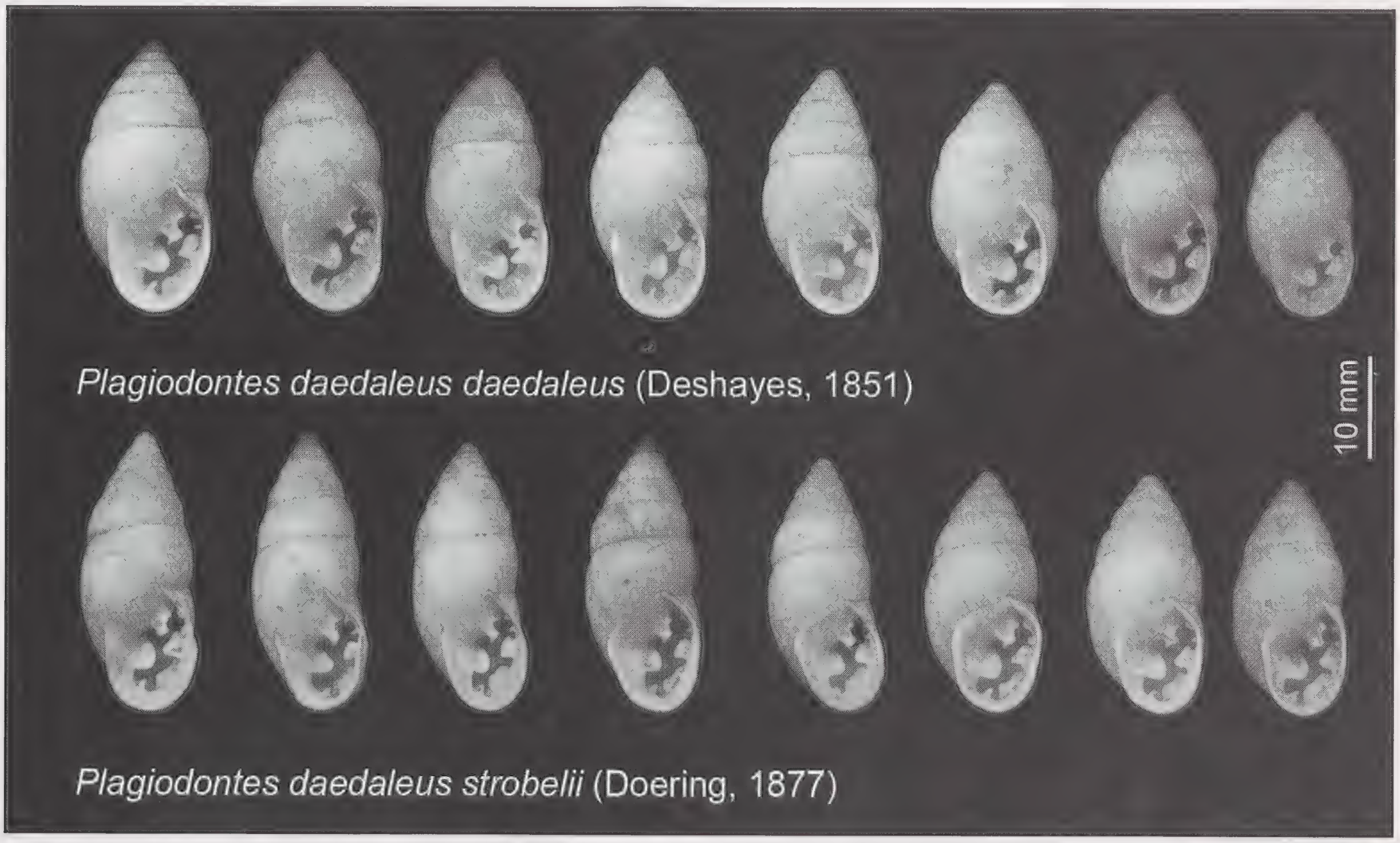

Plagiodontes daedaleus daedaleus (Deshayes, 1851)
10 mm
Plagiodontes daedaleus strobelii (Doering, 1877)

FIG. 4. Shell variability of the two species of *Plagiodontes* Doering, 1877, merged under the name *P. daedaleus* (Deshayes, 1851) in the literature.

TABLE 2. Summary of the shell variables (in mm) and proportions of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*. Mean ± standard deviation [coefficient of variation] (minimum-maximum). Independent samples t−test: all mean differences resulted in $p << 0.001$. Abbreviations as in Table 1.

Variable/ proportion	*P. daedaleus strobelii* (n = 248)	*P. daedaleus daedaleus* (n = 360)
SL	27.52 ± 2.110 [0.077] (21.70–33.53)	24.43 ± 2.124 [0.087] (17.46–30.90)
SW	12.46 ± 0.992 [0.08] (9.72–15.38)	12.83 ± 1.071 [0.084] (9.91–17.50)
LWL	18.24 ± 1.088 [0.06] (15.22–21.25)	17.04 ± 1.378 [0.081] (12.74–21.63)
LWW	11.58 ± 0.723 [0.062] (9.72–13.46)	12.16 ± 0.914 [0.075] (9.16–15.50)
AL	12.03 ± 0.829 [0.069] (9.86–14.63)	11.74 ± 1.030 [0.088] (9.12–15.50)
AW	9.21 ± 0.798 [0.087] (5.57–11.38)	9.75 ± 0.875 [0.090] (6.50–12.63)
AML	12.24 ± 0.830 [0.068] (10.00–14.88)	11.98 ± 1.030 [0.086] (8.89–16.13)
MA	124.38 ± 3.955 [0.032] (114.00–138.00)	117.71 ± 3.84 [0.033] (102.00–131.00)
SA	43.90 ± 5.083 [0.116] (27.00–57.00)	56.44 ± 5.24 [0.093] (42.00–76.00)
NW	7.36 ± 0.462 [0.063] (6.00–8.50)	7.25 ± 0.349 [0.048] (5.75–8.25)
Size (SL+SW)	39.99 ± 2.815 [0.070] (31.42–47.50)	37.26 ± 2.98 [0.080] (29.39–48.00)
SW/SL	0.45 ± 0.032 [0.070] (0.38–0.57)	0.53 ± 0.035 [0.066] (0.43–0.69)
LWL/SL	0.66 ± 0.028 [0.042] (0.56–0.73)	0.70 ± 0.025 [0.035] (0.61–0.76)
AL/SL	0.44 ± 0.024 [0.054] (0.36–0.50)	0.48 ± 0.024 [0.050] (0.42–0.56)
AW/AL	0.77 ± 0.049 [0.065] (0.54–0.86)	0.83 ± 0.043 [0.050] (0.69–1.01)

by growth lines on the remaining whorls; some shells have only growth lines (Figs. 3, 8).

The protoconch of *P. daedaleus strobelii* also has 2–2.5 whorls; the first half whorl has an irregular sculpture of spiral lines and fragments of axial components (Fig. 10), while the remaining two nepionic whorls are regularly sculptured with almost straight striae crossed by spiral lines (Fig. 11). The boundary with the teleoconch is a gradual transition to straight striae of variable thickness, with no spiral lines (Fig. 10). The teleoconch sculpture varies among different populations, exhibiting from weak growth lines to well-marked ribs (Figs. 12, 13).

Aperture Folds and Lamellae: All the studied shells of both subspecies had 9–14 apertural barriers (lamellae + folds) of similar distribution and shape (Figs. 14, 15).

The transverse lamella was centrally depressed in all the studied specimens, and

TABLE 3. Summary of shell and anatomical differences between *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii.*

	P. daedaleus daedaleus	*P. daedaleus strobelii*
Shell shape	ovate-elongate, obese	elongate-ovate, slender
Spire	blunt	acute
Protoconch-teleoconch boundary	well defined	gradual
Teleoconch sculpture	variable, generally smooth	variable, generally with strong axial ribs
Vagina shape	distally swollen	centrally swollen
Vaginal internal surface	reticulated	variable, generally principal longitudinal lamellae with few anastomoses to reticulated
Penis shape	club shaped	subcylindrical
Accessory lobe of the penial papilla	present	absent

generally longer in *P. daedaleus daedaleus* than in *P. daedaleus strobelii*. The tongue-shaped columellar lamella, the outer border of which is thick and slightly elevated, is the largest piece occluding the aperture in the two species. In *P. daedaleus daedaleus*, the columellar lamella is concave, with a rounded outline; its size is positively correlated to the

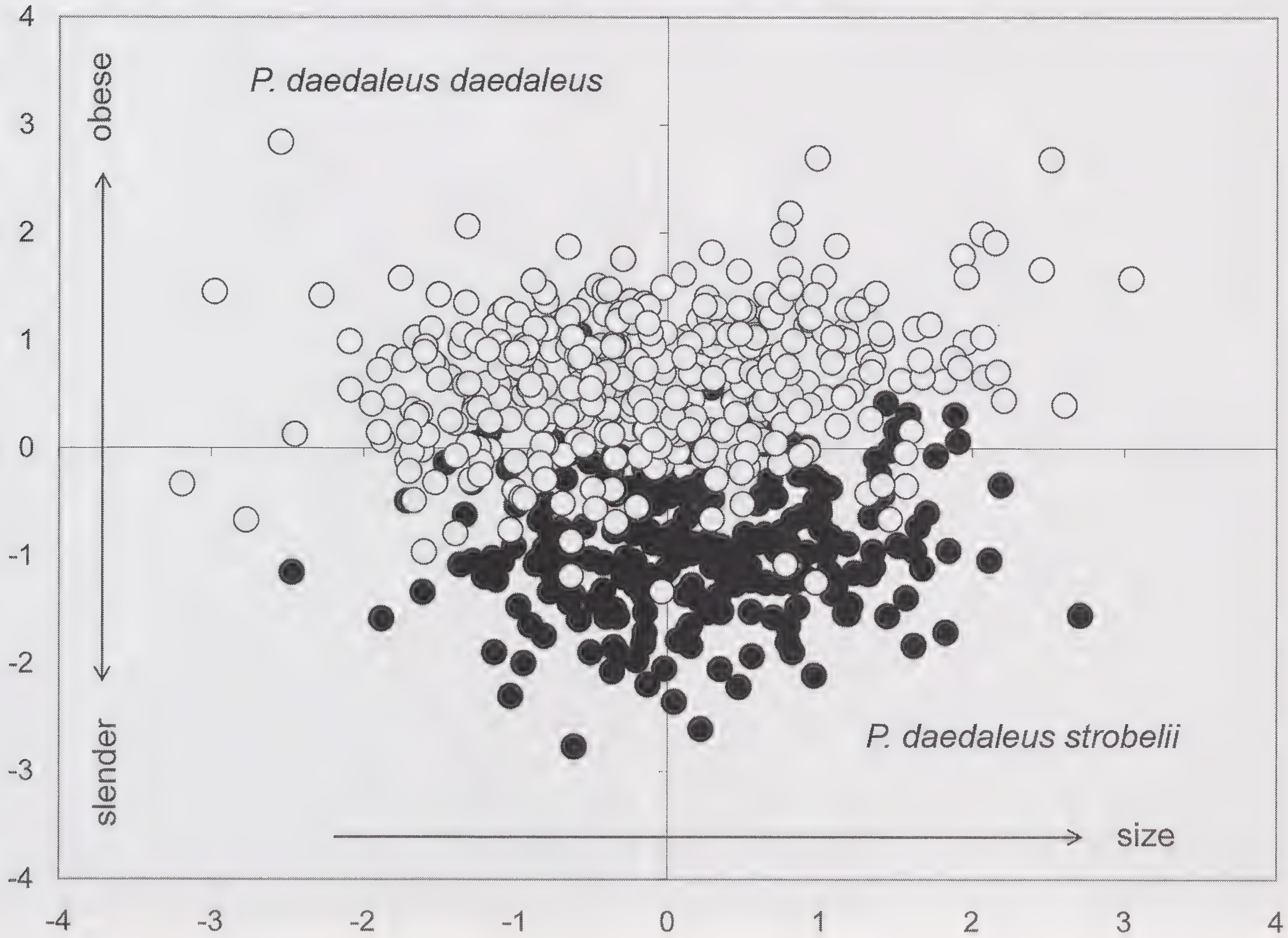
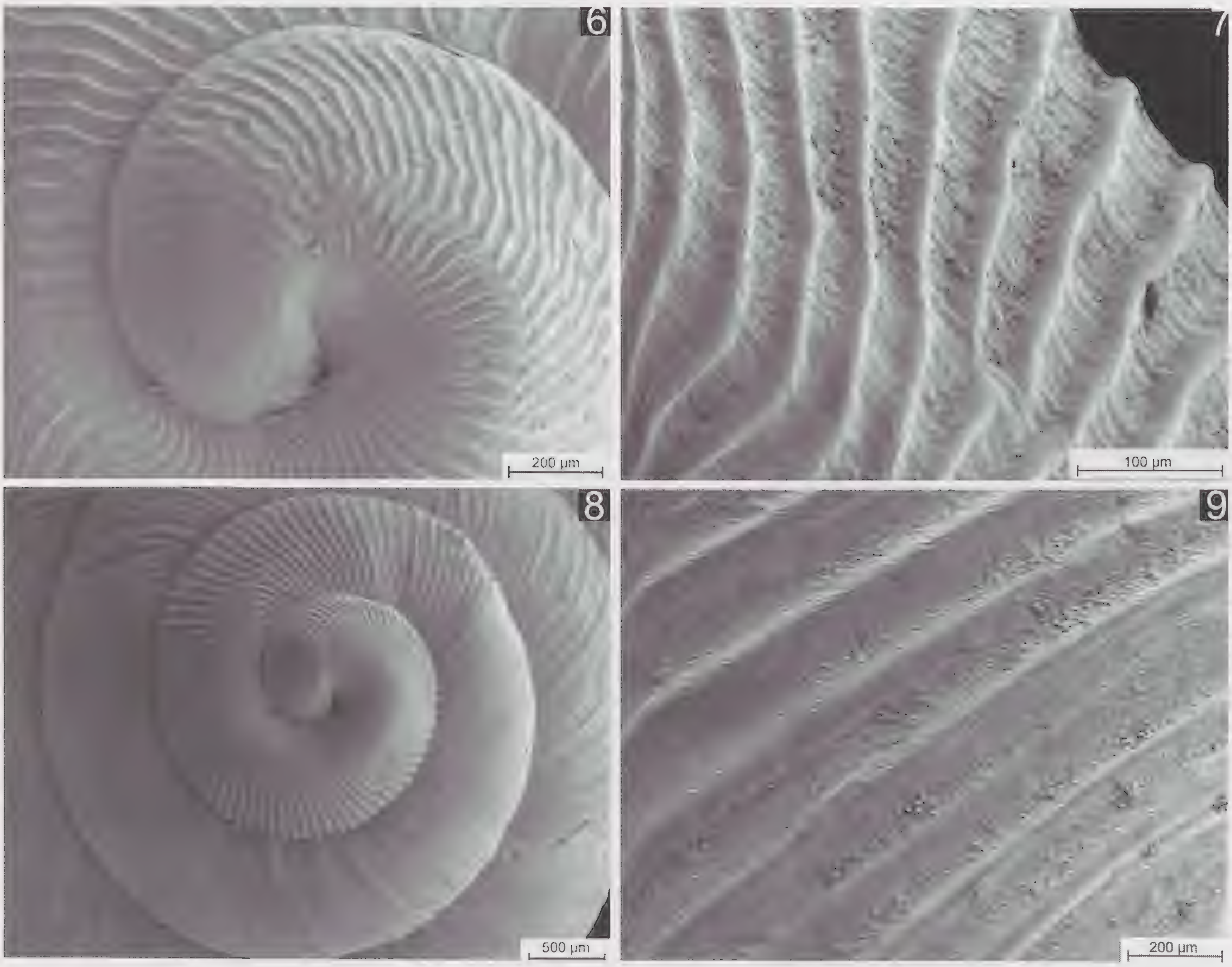


FIG. 5. Scatter plot of the scores of the first (PC1) and second (PC2) principal components performed on shell measurements of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*.

aperture width ($r = 0.650$; $n = 229$, $p < 0.01$); such a correlation was lower, but still significant in *P. daedaleus strobelii* ($r = 0.385$; $n = 95$, $p < 0.01$), the columellar lamella of which is relatively more straight and less concave than in *P. daedaleus daedaleus*. A minute supracolumellar knob or denticle of variable development may appear on the columellar lamella (Fig. 15). It was equally present in both *P. daedaleus daedaleus* (14.56% of the specimens; $n = 360$), and *P. daedaleus strobelii* (14.85% of the specimens; $n = 248$). The parietal lamella is dihedral, its faces forming a C to L profile; it is seen as a quadrangular plate when viewed from the apertural plane and it was united to the angular fold in all the studied specimens. The externally visible part of the parietal lamella is longer in *P. daedaleus daedaleus* than in *P. daedaleus strobelii*. Two suprapalatal folds were present in all the studied material; the upper one is usually triangular, though it is compressed in some specimens of *P. daedaleus daedaleus;* the lower one is always compressed. The upper-palatal lamella is constant in size and shape for both species. It is rectangular, with the outer border thickened and elevated; the lateral borders are curved downwards. The lower-palatal folds were present in most specimens, in numbers ranging from one to three or four, as shown in Figure 16. The lower-palatal fold located next to the upper-palatal lamella is usually the most developed one (principal lower-palatal). There was a very highly significant difference in the frequency distribution of the number of lower-palatal folds between *P. daedaleus daedaleus* and *P. daedaleus strobelii* ($\chi^2 = 23.90$; df =



FIGS. 6–9. SEM micrographs of the protoconch sculpture in *Plagiodontes daedaleus daedaleus*. FIG. 6: Axial undulated and anastomosed ribs of the first protoconch whorl; FIG. 7: Close-up of (6) showing spiral lines crossing axial striae; FIG. 8: Protoconch and first teleoconch whorls of *P. daedaleus daedaleus* (limit indicated by an arrow); FIG. 9: Teleoconch sculpture of *P. daedaleus daedaleus*.

4; p = 0.001). The number of lower-palatal folds was not correlated to size in either species (Fig. 17). The basal fold is laterally compressed and generally has the same size as the principal lower-palatal fold; it may be larger than the principal lower-palatal fold in *P. daedaleus strobelii*.

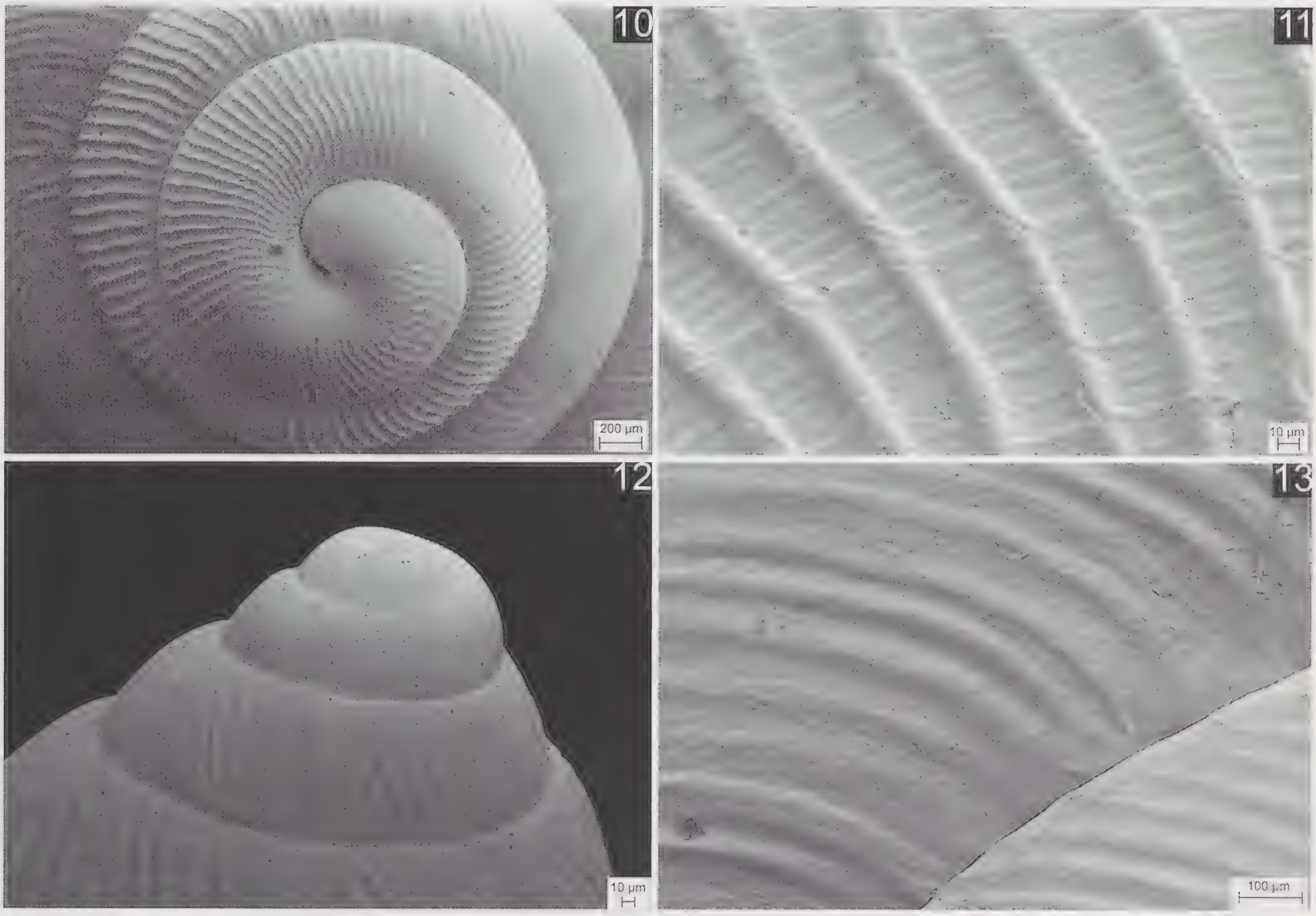
Comparative Anatomy (Table 3)

Pallial Complex: The length of the pallial complex of *P. daedaleus daedaleus* ranged between 19.5 mm and 39.5 mm long (29.8 mm on average) (Figs. 18, 19). The pallial complex of *P. daedaleus strobelii* (Figs. 20, 21) was within the same range, from 20.3 to 38.1 mm long (30.1 mm on average).

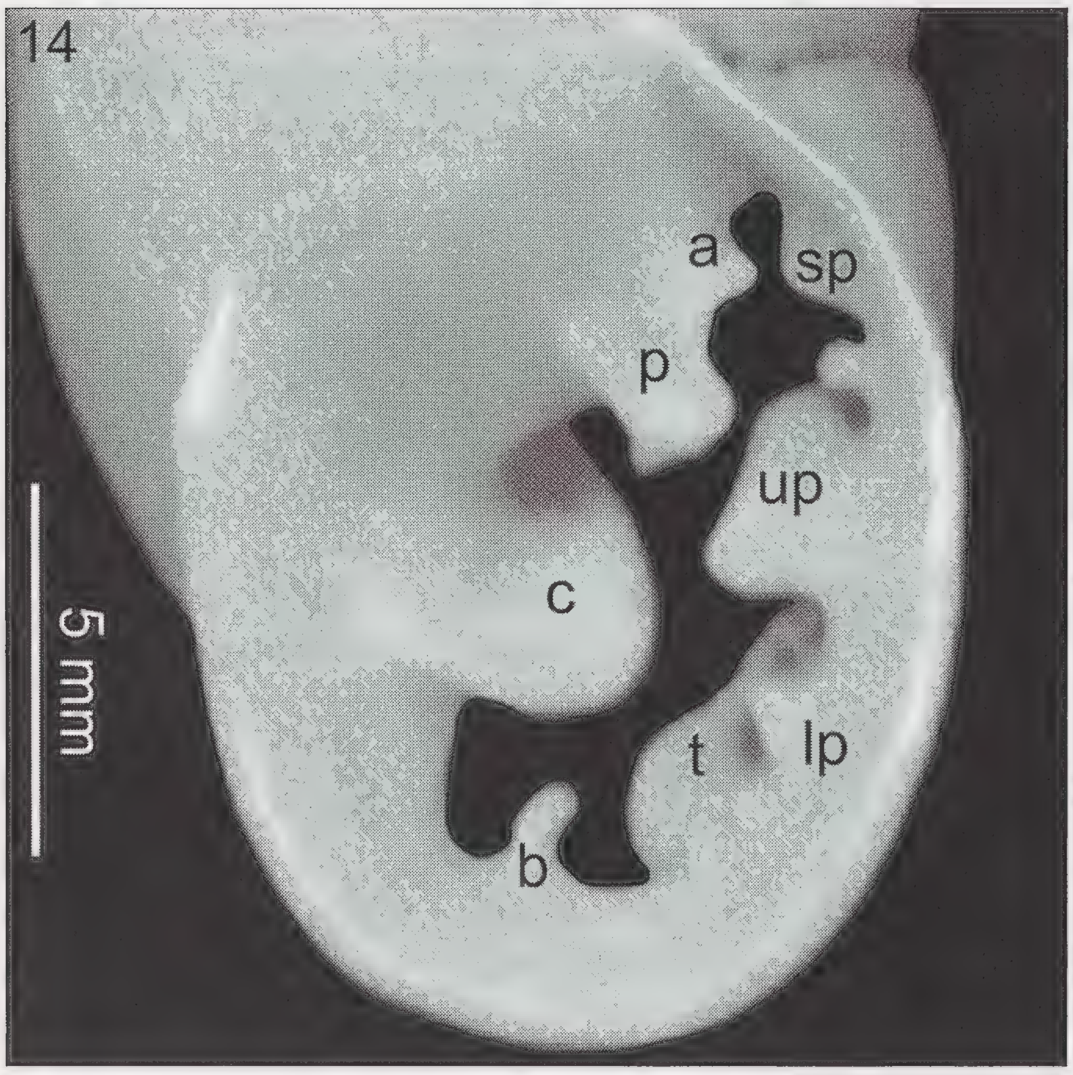
The nearly triangular kidney was always located in the proximal part of the lung cavity alongside the periaortic intestinal bend. The kidney of *P. daedaleus daedaleus* is twice as long as it is wide and occupies between 20% and 50% of the length of the lung; the kidney of *P. daedaleus strobelii* is usually shorter, only 1.6 times as long as it is wide, and it never occupied more than 25% of the length of the lung.

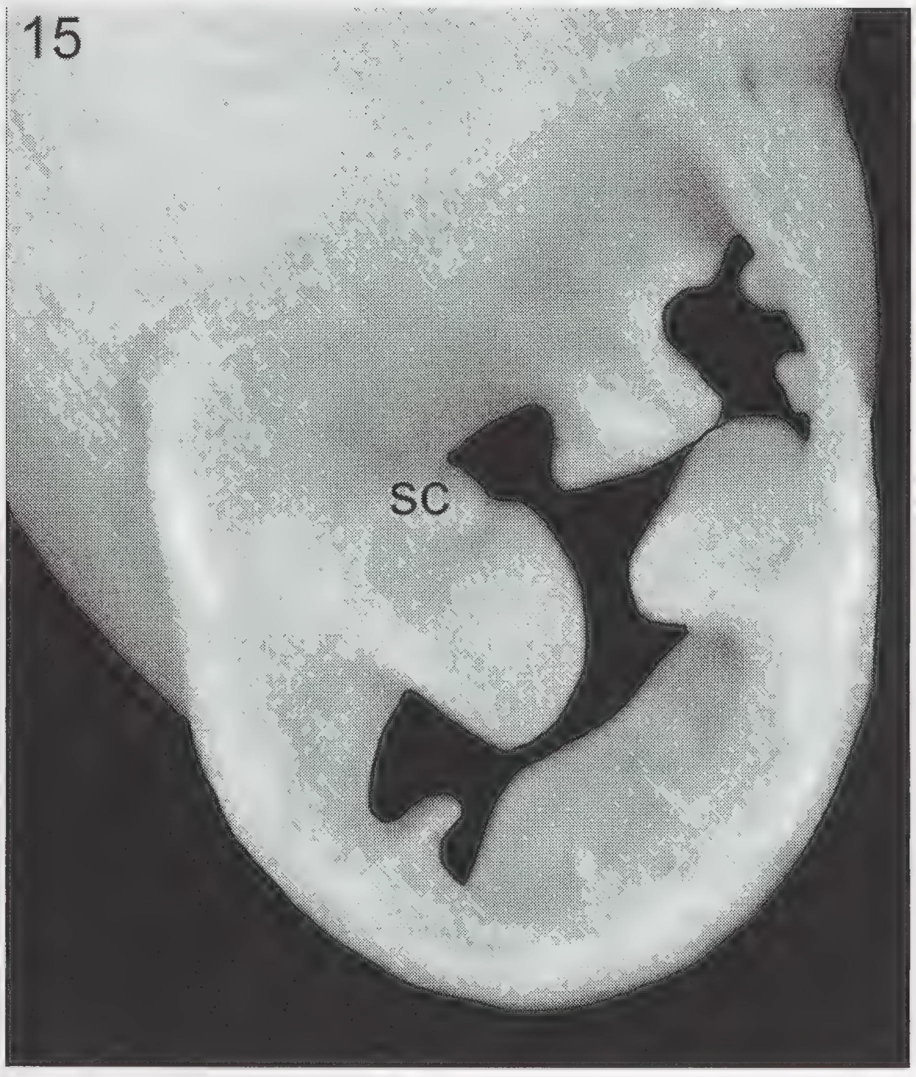
No other anatomical differences were found between the pallial complex of the two taxa. The internal structure of the kidney was similar in all the studied material, with two layers of longitudinal lamellae, the upper layer of which showed short, thin straight lamellae, while the lower one was formed by long and thicker undulating lamellae. The primary ureter runs along the rectal side of the kidney up to the top of the lung cavity; then it turns down along the rectum and forms the secondary ureter, which opens at the ureteric pore, at the level of the midpoint of the kidney. From that point onwards, the open secondary ureter is delimited by two ridges that form a ureteric groove ending in the pneumostome. The rectal ridge is the less developed one in both species.

The pericardium, located in the proximal side of the pallial complex, was variable in



FIGS. 10–13. SEM micrographs of the protoconch sculpture in *Plagiodontes daedaleus strobelii*. FIG. 10: Axial undulated and anastomosed ribs of the first protoconch whorl; FIG. 11: Close-up of (10) showing spiral lines crossing axial striae; FIG. 12: Protoconch and first teleoconch whorls; FIG. 13: Teleoconch sculpture.





FIGS. 14–15. Aperture and apertural lamellae and folds. FIG. 14: *Plagiodontes daedaleus strobelii*; FIG. 15: *P. daedaleus daedaleus*. Abbreviations: a, angular fold; b, basal fold; c, columellar lamella; lp, lower-palatal fold; p, parietal lamella; up, upper-palatal lamella; sc, supracolumellar knob; sp, suprapalatal folds; t, transverse lamella.

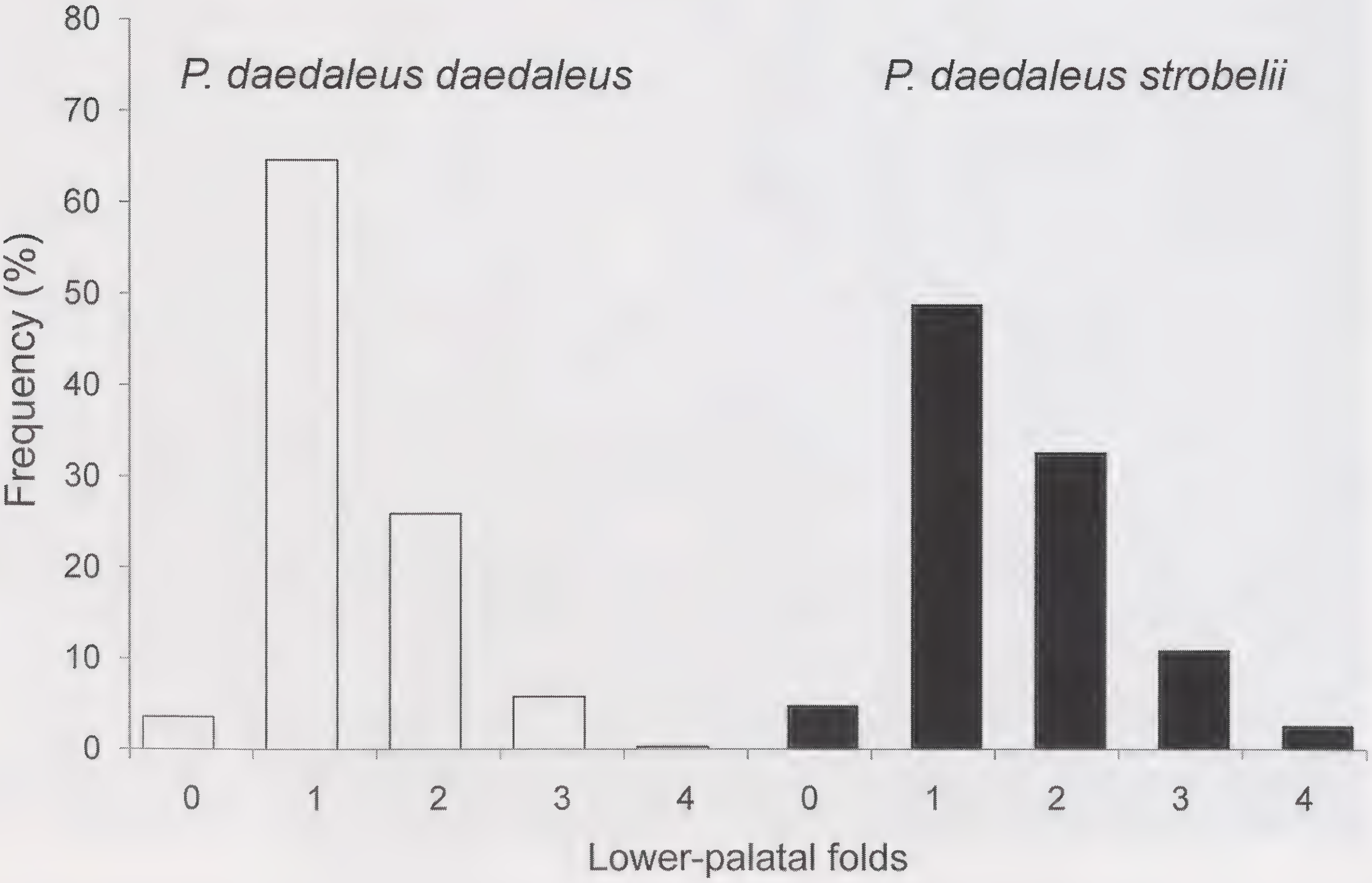


FIG. 16. Frequency distribution of the number of lower-palatal folds in *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*.

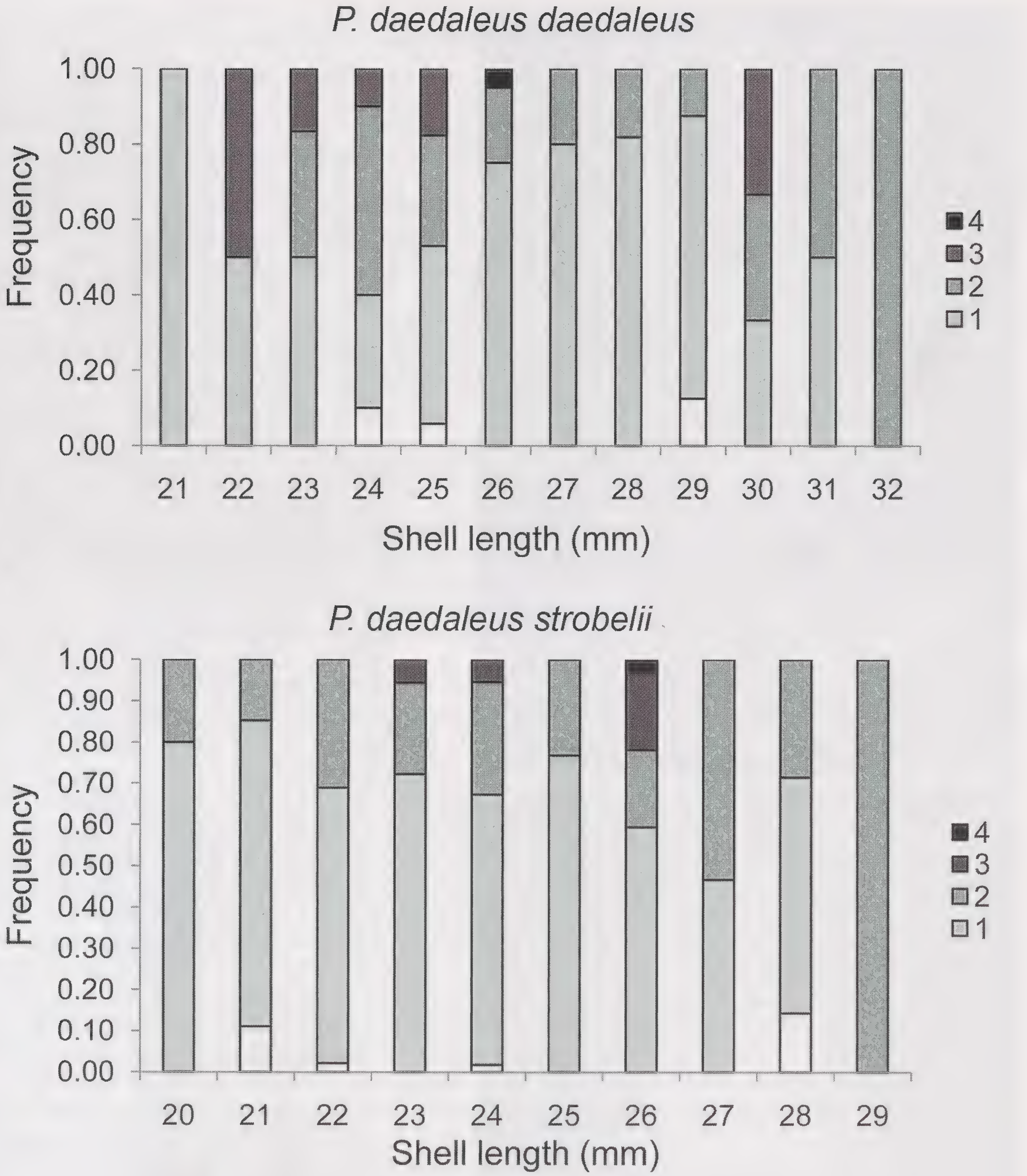


FIG. 17. Frequency of shells of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii* showing either 0, 1, 2, 3 or 4 lower palatal teeth plotted against shell length.

length, though of similar size in both species (3.0–5.4 mm in *P. daedaleus daedaleus*; 3.5–6.0 mm in *P. daedaleus strobelii*); it is equivalent to 50–60% of the kidney length.

A prominent pulmonary vein runs parallel to the rectum and reaches the mantle collar. The pulmonary vein was 18–29 mm long in *P. daedaleus daedaleus*, and 14–30 mm long in *P. daedaleus strobelii*.

The afferent marginal vein of *P. daedaleus strobelii* branches near the midpoint of the pulmonary vein, thus equaling from 40% to 62% of the length of the latter; this position is quite variable in *P. daedaleus daedaleus,* where the afferent marginal vein was 39% to 75% of the pulmonary vein length.

All the studied material showed a conspicuous vascularization between the secondary

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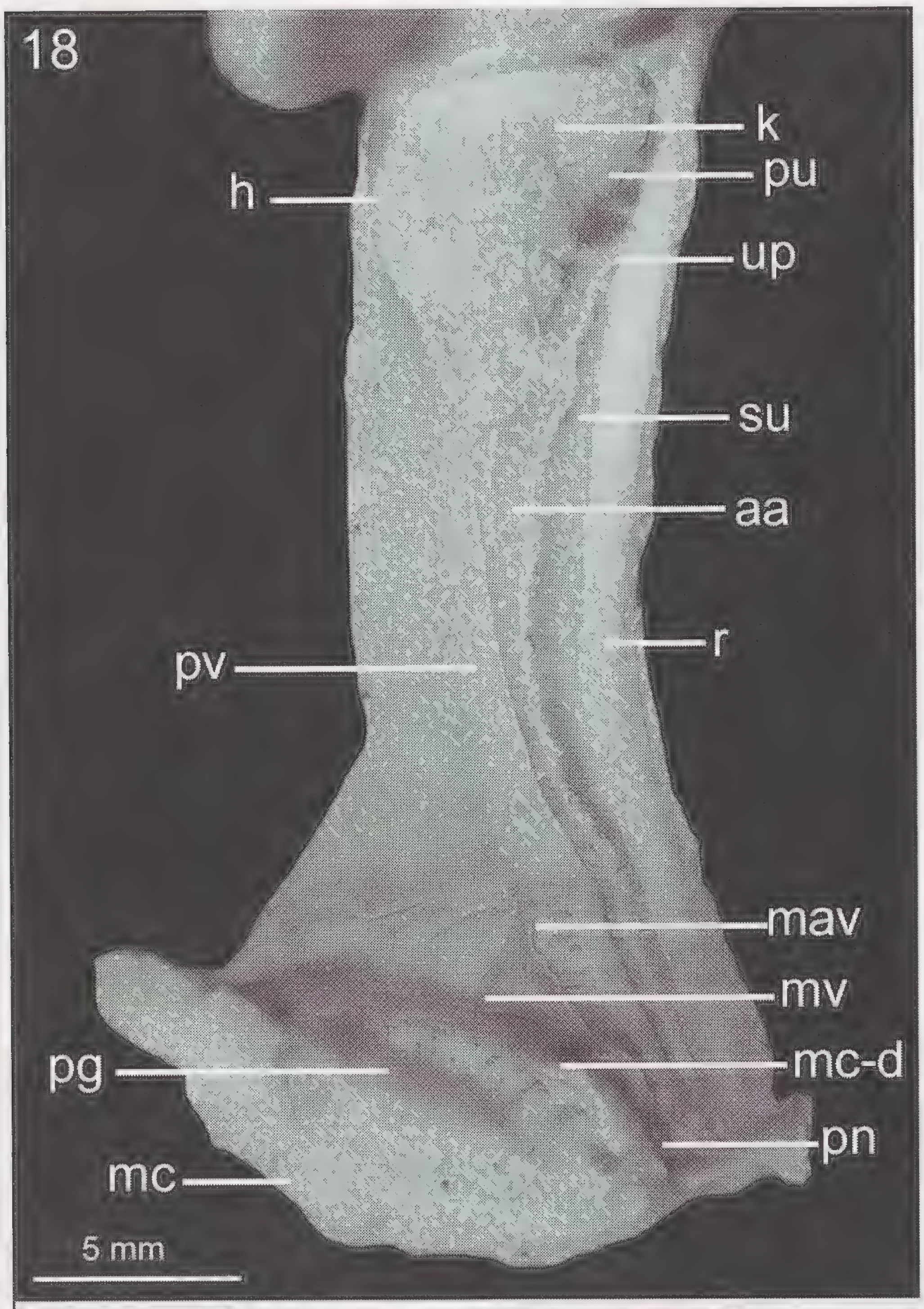
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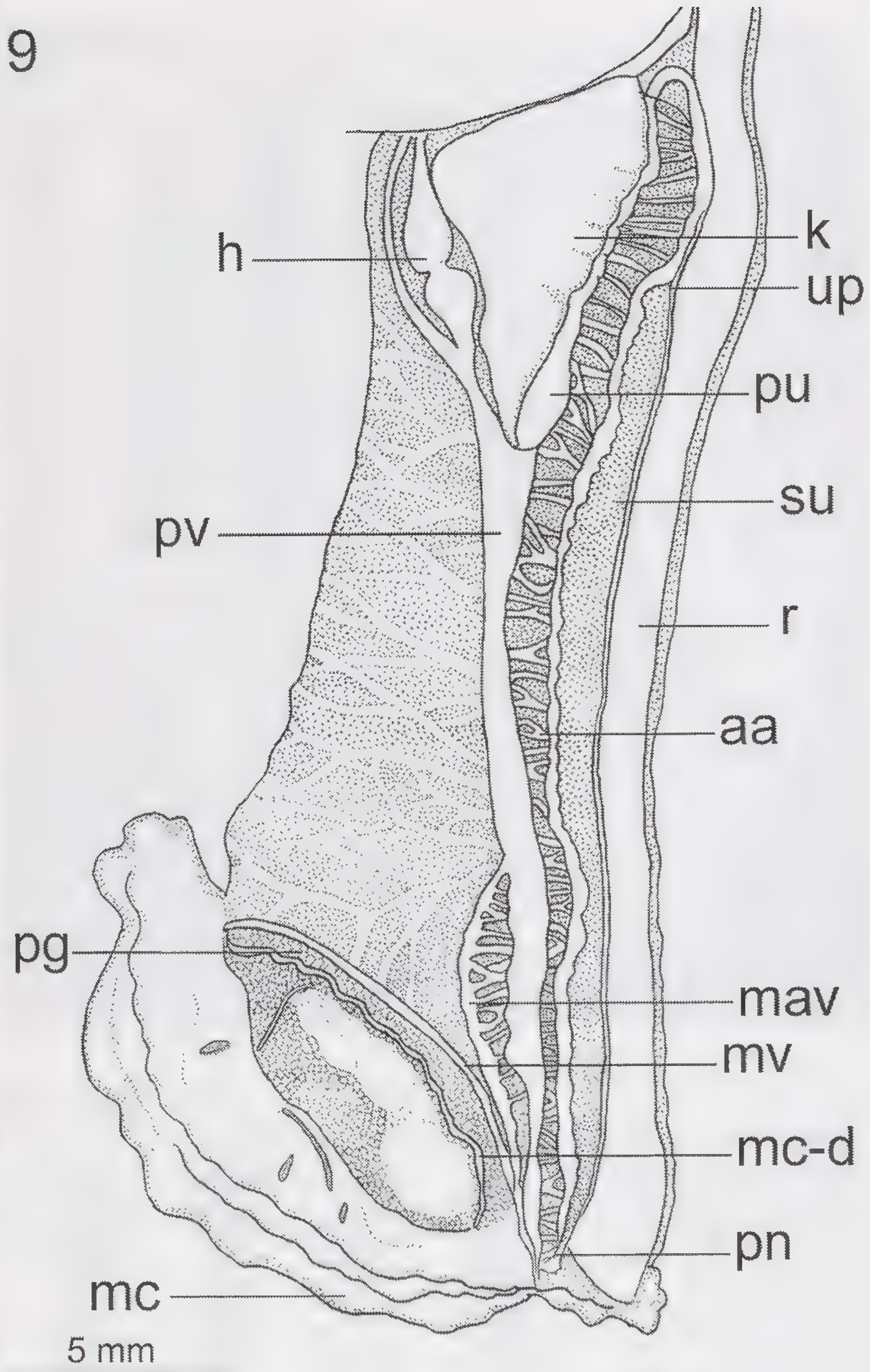
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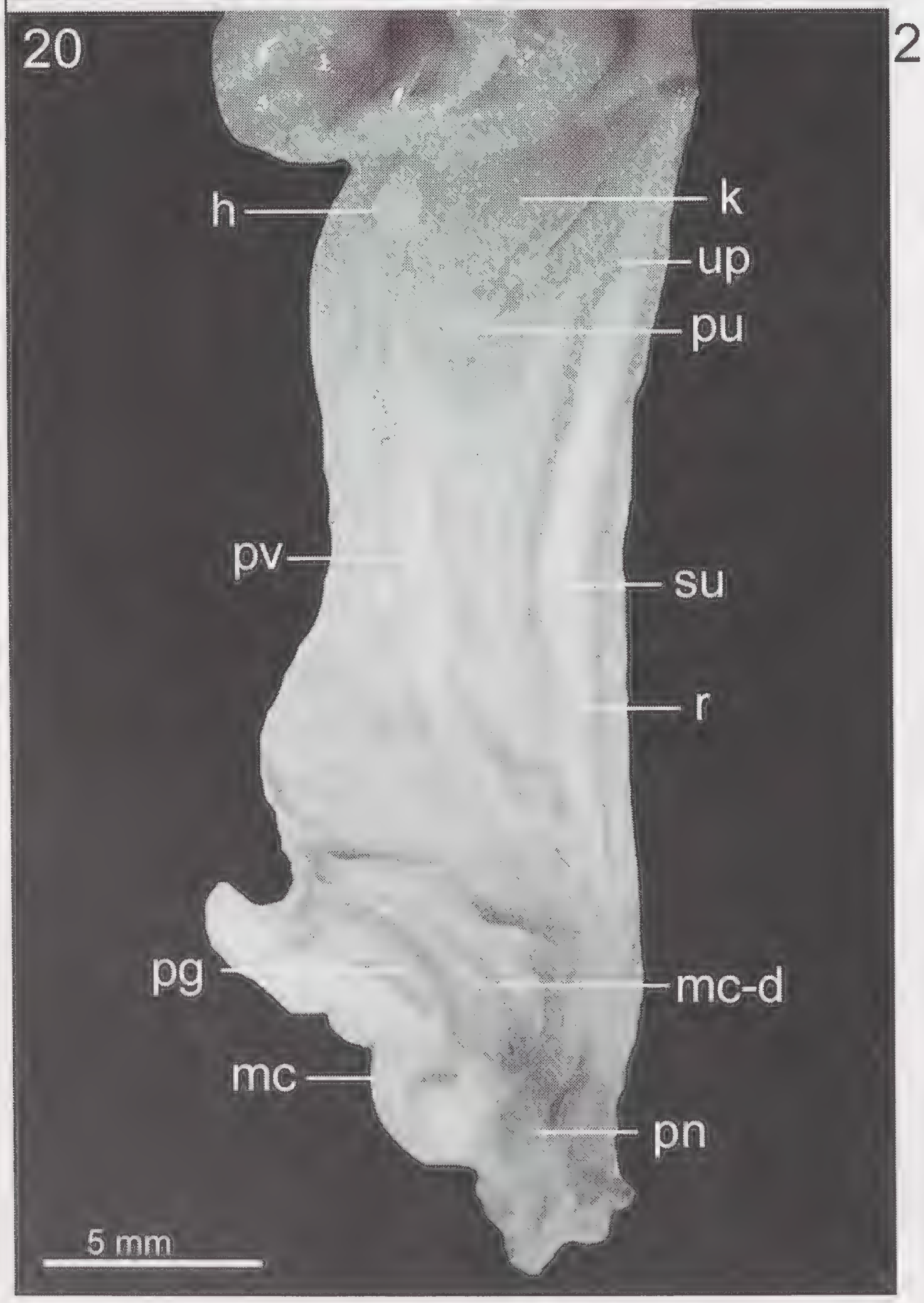
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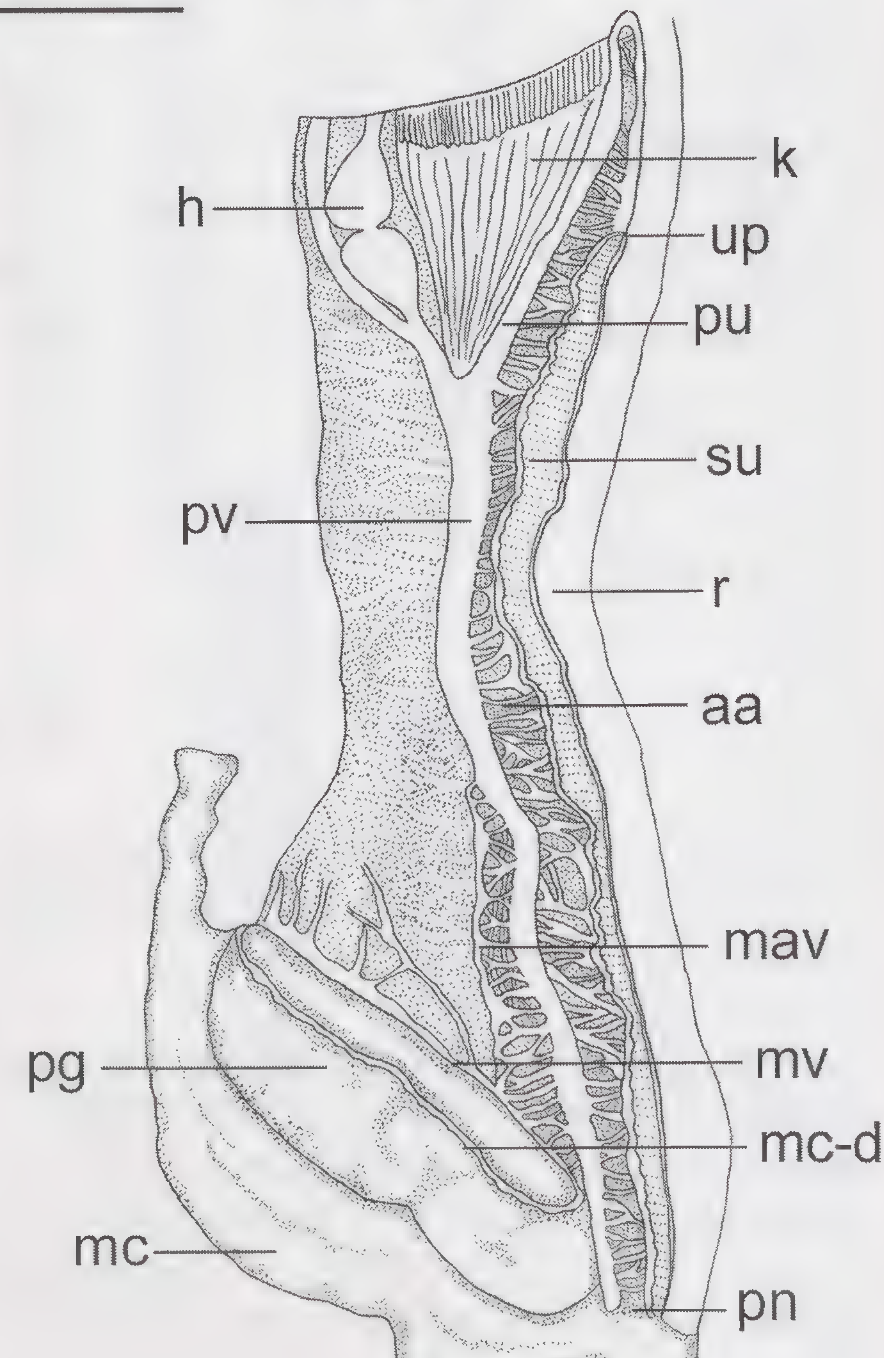
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ureter and the pulmonary vein (ad-rectal area), and between the pulmonary vein and the marginal afferent vein. A marginal vein branching from the distal portion of the pulmonary vein and running along the mantle collar was always present. The region of the lung delimited by the pericardium, the columellar side and the above mentioned vessels (pulmonary, marginal afferent and marginal vein) was translucent and exhibited slight vascularization.

The mantle collar includes a brown, spongy pallial gland and some indentations corresponding to the position of the apertural lamellae and folds.

Reproductive System: The genital organs of *P. daedaleus daedaleus* are illustrated in Figures 22–24. The ovotestis, embedded in the digestive gland, is composed of 6–7 groups of digitiform acini (most often six) that converge to form the collector duct. The studied specimens of *P. daedaleus strobelii* (Figs. 25–27) had six groups of acini.

Ovotestis color is variable (white, orange or light brown) in *P. daedaleus daedaleus*, sometimes with black spots; it is orange to light or reddish-brown in *P. daedaleus strobelii*.

The hermaphroditic duct was similar in all the studied material. It is brown, markedly convoluted, and runs along the columellar side of the visceral mass. The diameter of the duct varied among specimens, but its central portion always became dilated to form the vesicula seminalis.

The albumen gland is elongated, highly variable in size and color (white or pale orange in *P. daedaleus daedaleus;* white, cream or pale orange in *P. daedaleus strobelii*), and located against the anterior, concave surface of the digestive gland over the digestive pouch. The fertilization pouch-spermathecal complex (FPSC) is white, conspicuously visible on the basal side of the albumen gland. FPSC is proximally swollen, while its distal part consists of a free blind sac.

The spermoviduct is composed of a hyaline pale-orange oviduct portion (uterus) and a white and glandular prostatic portion. The uterus continues into the free oviduct that ends at the vagina, while the prostatic portion extends to the vas deferens.

The hermaphroditic duct, the albumen gland and the uterine part of the spermoviduct are highly variable in size. The penis retractor muscle is always attached to the penis-epiphallus boundary.

The bursa copulatrix of *P. daedaleus daedaleus* is generally a round sac, 1.20 to 3.10 mm in diameter, though it was slightly ovoid in some specimens; it is a round to ovoid sac in *P. daedaleus strobelii,* 1.76 to 2.84 mm in diameter. The color of the bursa varies, in both species, depending on the eventual presence of sperm, that is, it is white or cream color in virgin snails, and brown in the individuals that have already copulated.

The duct of the bursa copulatrix was 13 to 27 mm long in *P. daedaleus daedaleus,* and 17 to 27 mm long in *P. daedaleus strobelii*. Internally, it exhibits straight folds or lamellae in both species.

The penial complex, consisting of penis, epiphallus and flagellum, is long and occupies a high proportion of the anterior portion of the visceral cavity, with a total length of 26.2–47.6 mm in *P. daedaleus daedaleus* (Figs. 28–31), and of 26.2–39.2 mm in *P. daedaleus strobelii* (Figs. 32–35).

The main internal differences between *P. daedaleus daedaleus* and *P. daedaleus strobelii* were found in the anatomy of penis and vagina. The penis is club-shaped in *P. daedaleus daedaleus* (Figs. 22, 23), due to a significant swelling of its proximal portion, which was 2.6 to 4.2 mm wide; the distal part is much narrower and of variable length, and therefore the high variability of penis length (4.8 to 14.7 mm long) was mostly due to variations in the length of the narrow part. By contrast, in *P. daedaleus strobelii* the penis has only a slight swelling towards the epiphallus (Figs. 25, 26); as a result, the organ is almost cylindrical in shape and significantly thinner than in *P. daedaleus daedaleus* (1.89 to 2.62 mm wide). Penis length was also highly variable in *P. daedaleus strobelii,* 3.50 to 14.29 mm long, that is, in the same range of shell

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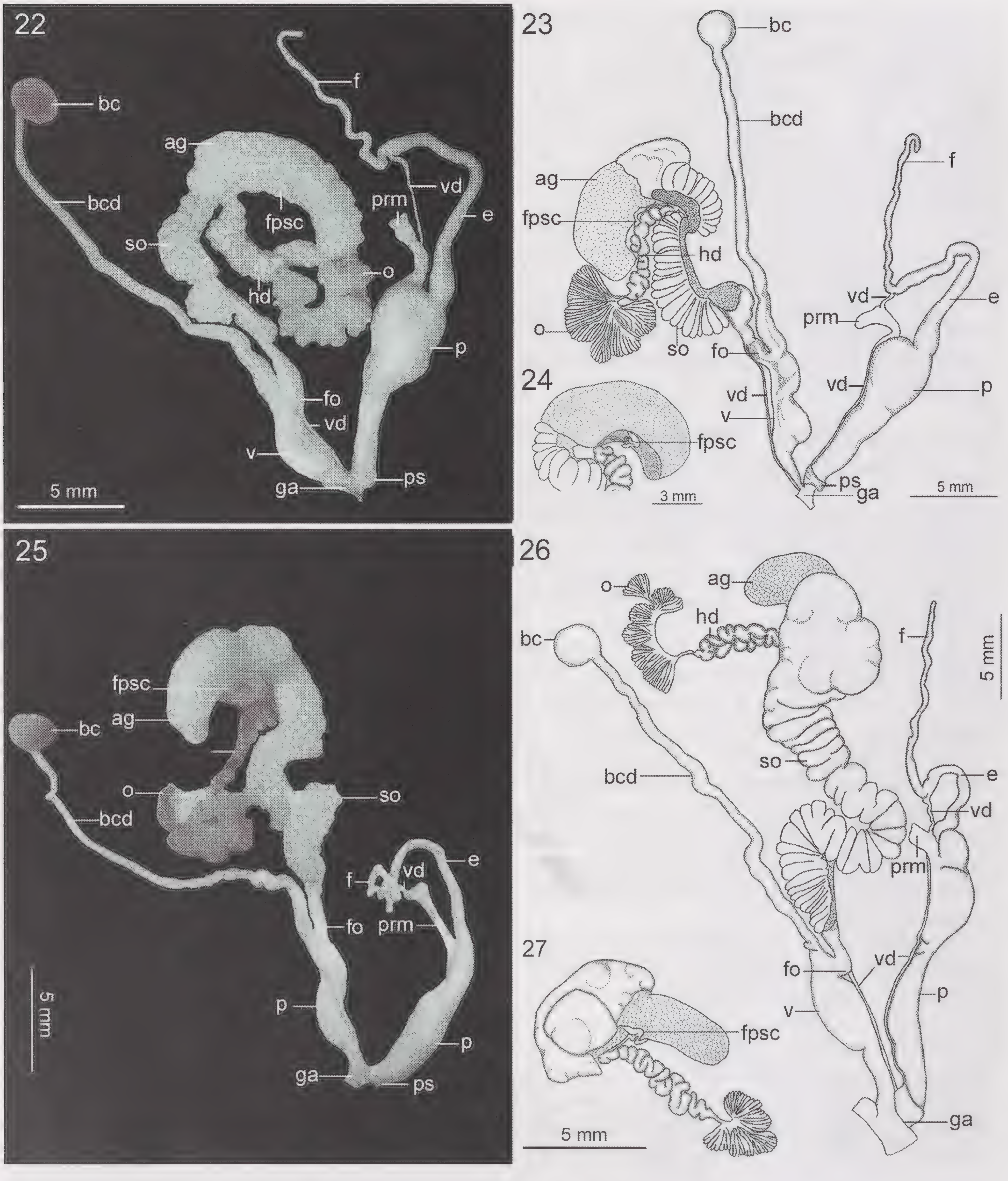
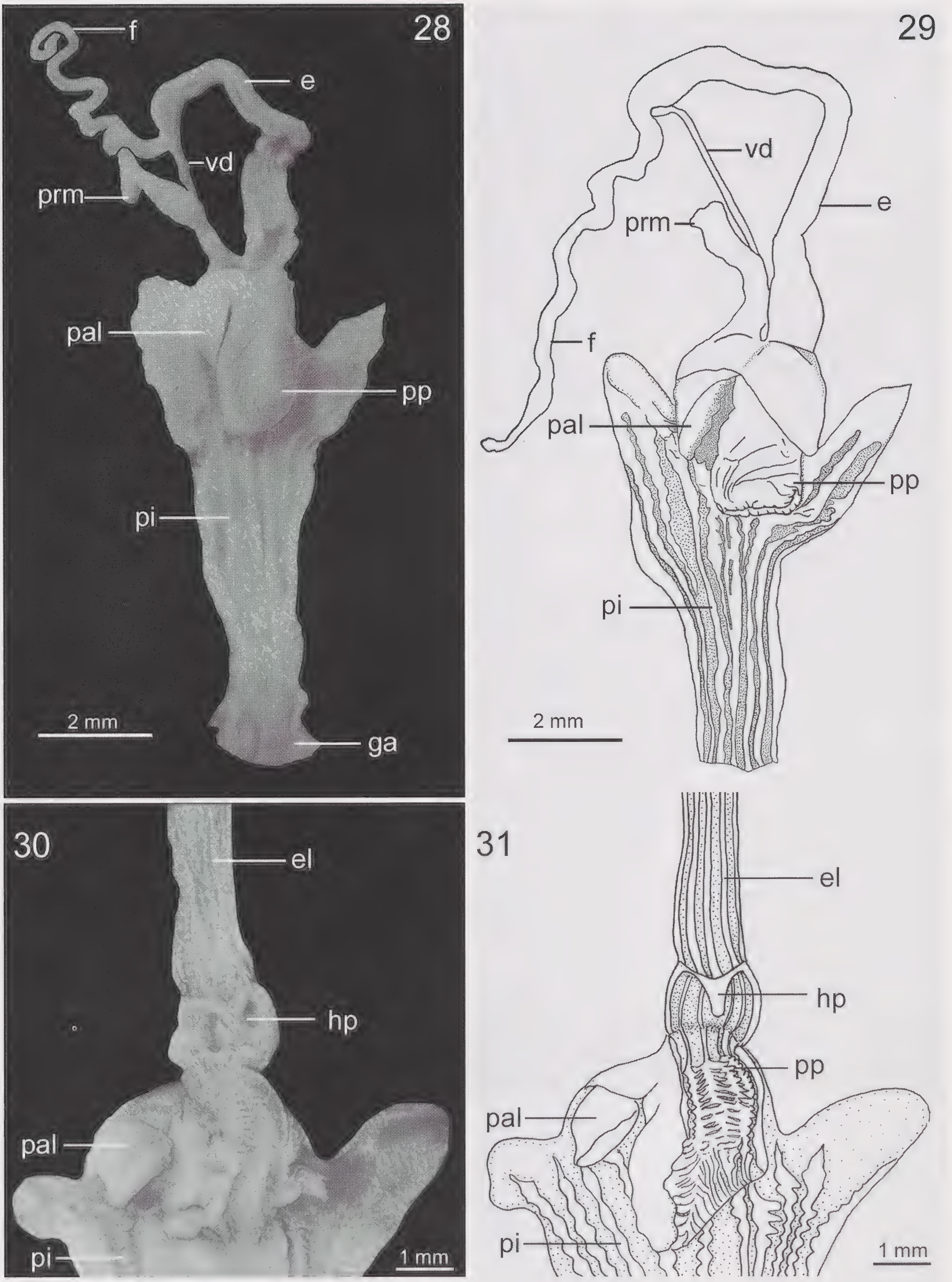
FIGS. 18–21. Pallial complex of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*. FIGS. 18, 19: Pallial complex of *P. daedaleus daedaleus*; FIGS. 20, 21: Pallial complex of *P. daedaleus strobelii*. Abbreviations: aa, adrectal area; h, heart; k, kidney; mav, marginal afferent vein; mc, mantle collar; mc–d, mantle collar and diaphragm junction; mv, marginal vein; pg, pallial gland; pn, pneumostome; pu, primary ureter; pv, pulmonary vein; r, rectum; su, secondary ureter; up, ureteric pore.



FIGS. 22–27. Genital system of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii.* FIGS. 22, 23: Genital system of *Plagiodontes daedaleus daedaleus*; FIG. 24: Proximal genitalia of *P. daedaleus daedaleus*; FIGS. 25, 26: Genital system of *Plagiodontes daedaleus strobelii*; FIG. 27: Proximal genitalia of *P. daedaleus strobelii.* Abbreviations: ag, albumen gland; bc, bursa copulatrix; bcd, bursa copulatrix duct; e, epiphallus; f, flagellum; fo, free oviduct; fpsc, fertilization pouch-spermathecal complex; hd, hermaphroditic duct; o, ovotestis; p, penis; prm, penis retractor muscle; ps, penial sheath; so, spermoviduct; v, vagina; vd, vas deferens.

length values of *P. daedaleus daedaleus*. The proportion penis-length/penis-width was 1.60 to 4.32 in *P. daedaleus daedaleus* (2.76 on average), whereas the penis was 2.75 to 6.00 times as long as it is wide in *P. daedaleus strobelii* (4.67 on average).



FIGS. 28–31. Terminal male genitalia of *Plagiodontes daedaleus daedaleus*. FIGS. 28, 29: Internal structure of penis; FIGS. 30, 31: Internal structure of epiphallus and penial papilla. Abbreviations: e, epiphallus; el, epiphallic lamellae; f, flagellum, ga, genital atrium; hp, hollow papilla of the epiphallus; pi, pilaster; pp, penial papilla; pal, penial papilla accessory lobe; prm, penis retractor muscle; vd, vas deferens.

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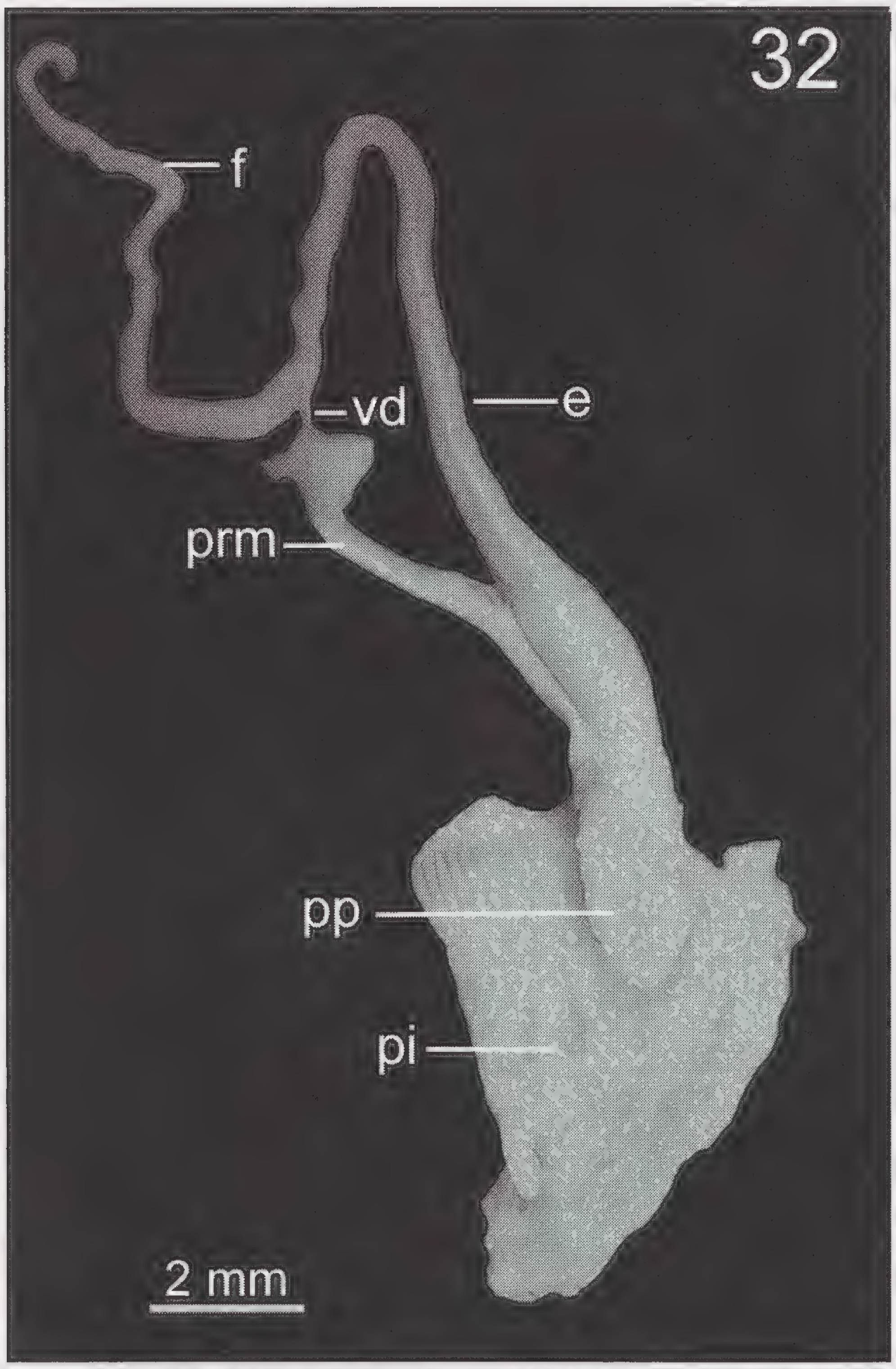

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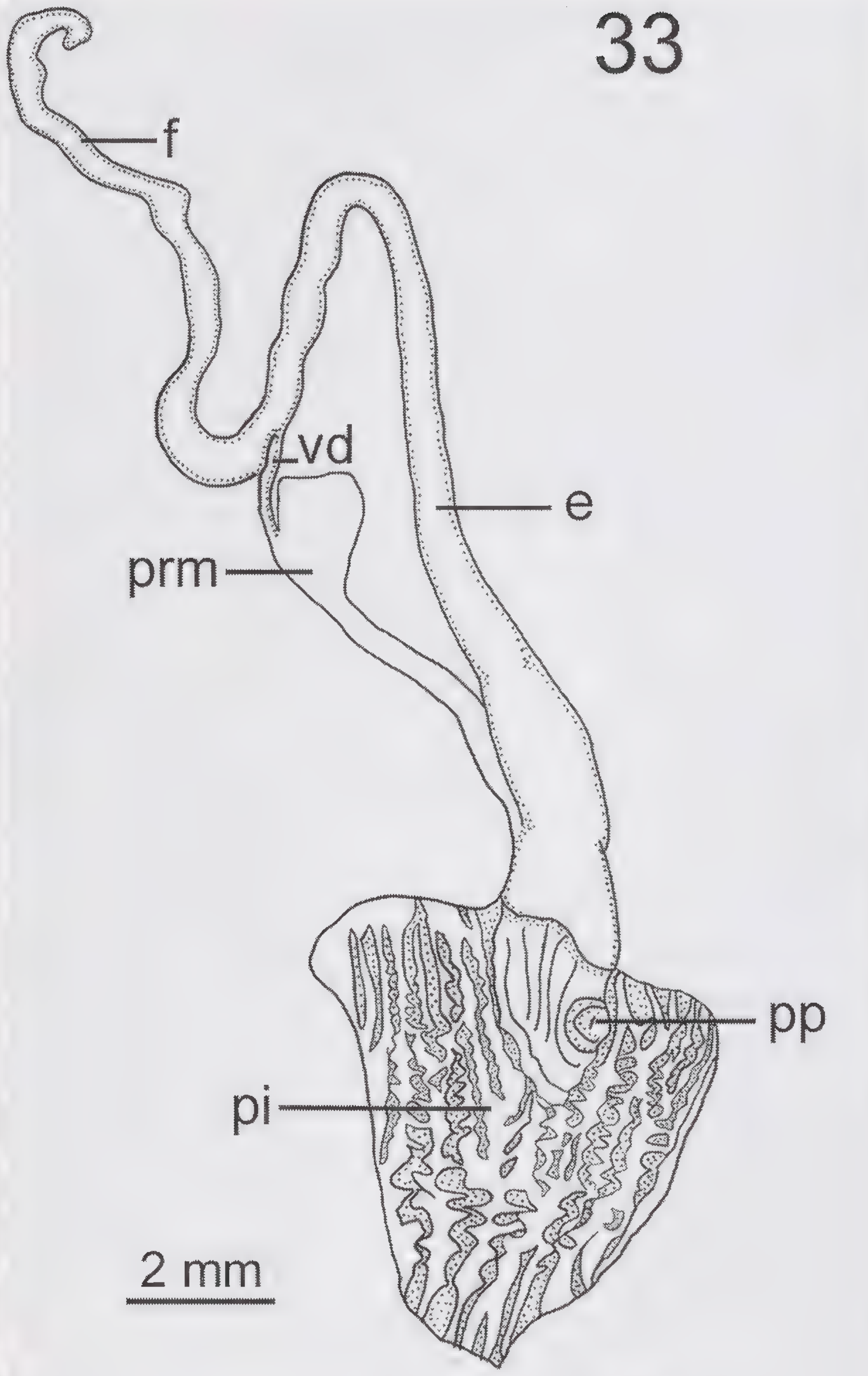

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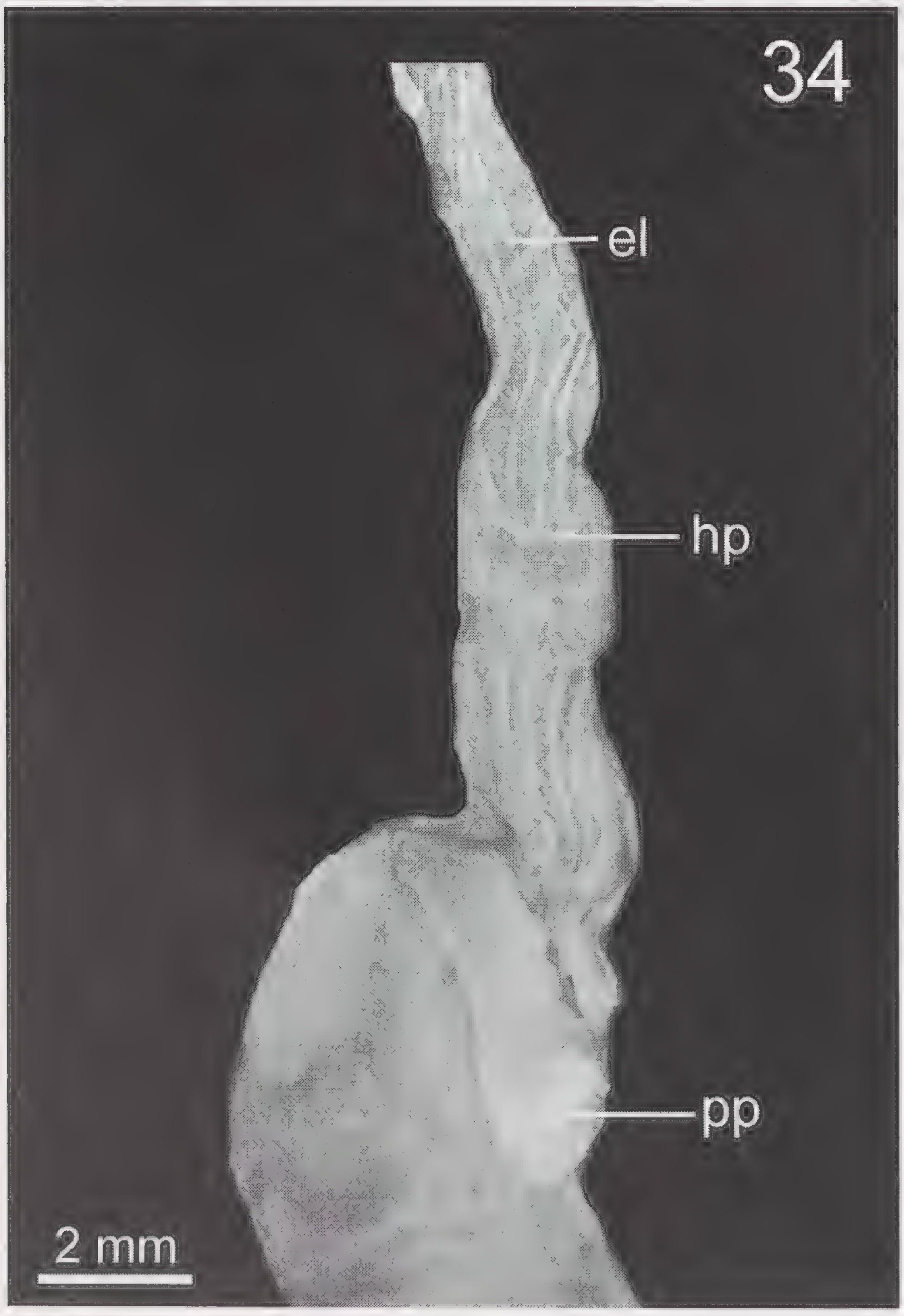

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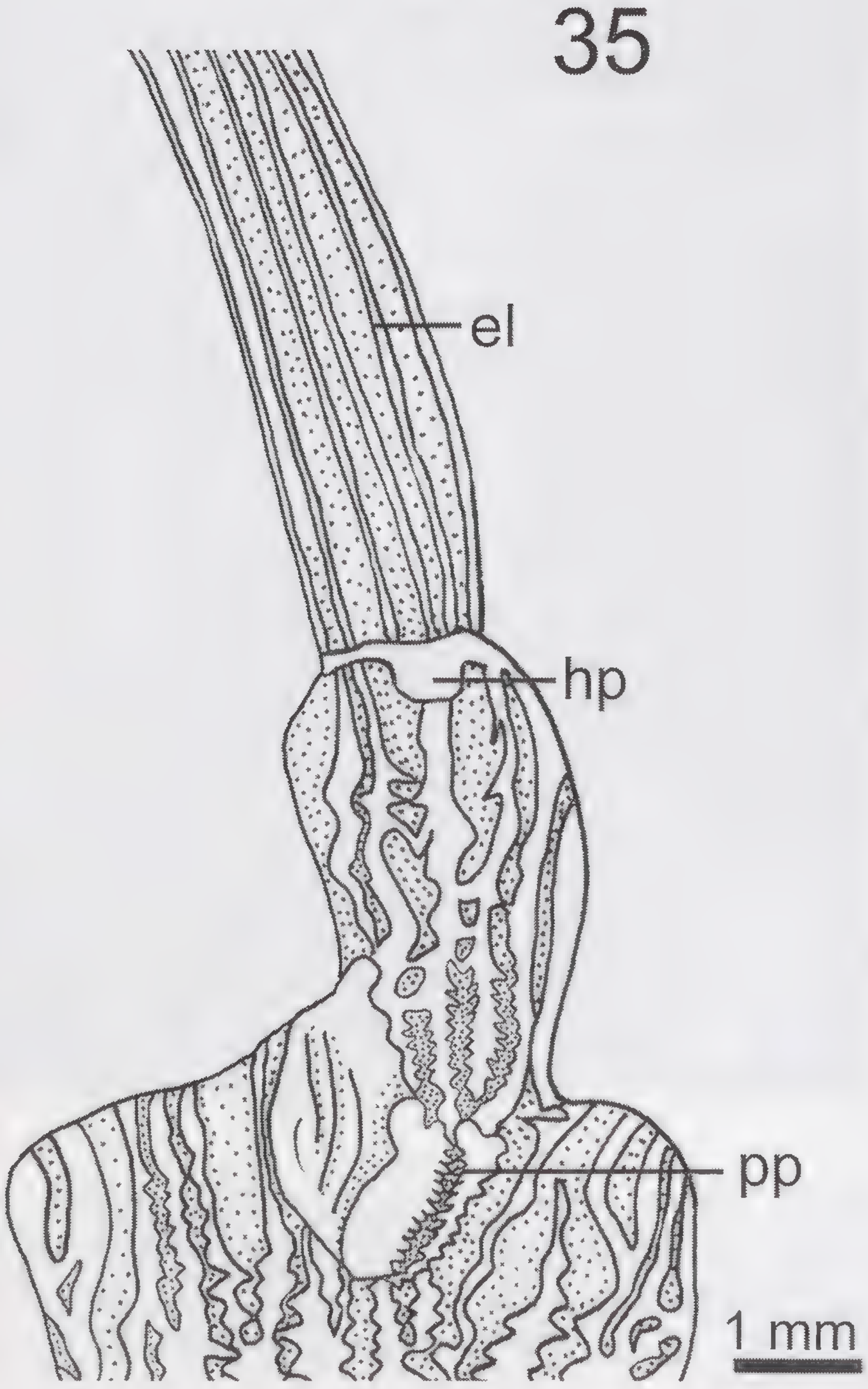

The external swelling of the penis is correlated with its inner anatomy. The verge exhibited an accessory lobe in *P. daedaleus daedaleus* (Figs. 28, 29), with an internal tube sculptured with anastomosing folds (Figs. 30, 31). In *P. daedaleus strobelii,* the penial papilla was quadrangular, elongated and simple, with no accessory lobe (Figs. 32, 33) but also with internal anastomosing folds (Figs. 34, 35).

The inner wall of the penis of *P. daedaleus daedaleus* exhibits longitudinal folds or pilasters of variable development, ranging from thin and straight to prominent and undulated folds. The pilasters always become thin and straight in the area surrounded by the penis sheath, and are absent underneath the papilla (Figs. 28, 29). In *P. daedaleus strobelii,* the internal surface of the penis is composed of pilasters with a variable degree of anastomosis, which become distally thin and straight (Figs. 32, 33).

The penis sheath is short in both species. The epiphallus is cylindrical, except for a swelling in the transition to the penis, which is marked by a constriction; it was 10.12–17.86 mm long (13.25 mm on average) in *P. daedaleus daedaleus,* and 8.93–18.94 mm long (12.38 mm long on average) in *P. daedaleus strobelii*. Internally, the cylindrical part exhibits five to seven straight folds in both species (Figs. 30, 31 and 34, 35), and it is separated from the swollen part by a septum-like structure at a point where the folds join to form a minute hollow papilla. The swollen part continues into a cylindrical tube that runs into the fleshy penial papilla. This tube has an internal sculpture of elevated, branching and anastomosing folds.

The vas deferens is a tubule of constant diameter that emerges exactly above the point where the vagina bifurcates to form the free oviduct and the bursa copulatrix duct. It is shorter than the sum of the lengths of the penis and the epiphallus. The vas deferens runs attached to the vagina, passes through the penis sheath, and continues along the penis, attached to its surface, to end finally in the epiphallus-flagellum boundary, after crossing the retractor muscle (Figs. 25, 26). The flagellum is cylindrical, as long as the epiphallus, and has one straight internal fold in both species; it was 10.12 to 17.86 mm long (13.25 mm on average) in *P. daedaleus daedaleus,* and 8.06 to 13.69 mm long (11.1 mm on average) in *P. daedaleus strobelii*.

The vagina of *P. daedaleus daedaleus* is generally slightly shorter than the penis, swollen and two to four times as long as it is wide; it was 4.74 to 10.82 mm long, 1.55 to 3.45 mm wide in the studied material. The inner vaginal surface is covered by anastomosing thin folds which give it a reticulated aspect (Figs. 36, 37).

The vagina of *P. daedaleus strobelii* has similar characteristics, except that it is centrally swollen; it was 6.30 to 8.33 mm long, 1.35 to 3.00 mm wide, and 2.4 to 5.0 times as long as it is wide. It exhibited a remarkable intra- and interpopulational variation (Figs. 38–40), with a sculpture of main longitudinal folds that may show from some minor transverse folds up to a true reticulation.

A genital atrium was almost absent in all the studied material, since vagina and penis join together at the genital pore.

TAXONOMY

On the basis of the anatomical differences detected between the snails living to the east and west of the Pampean Sierras, we have compiled a new synonymy for *P. strobelii*, as a species characterized by the following diagnosis.

ORTHALICIDAE Albers, 1860
Odontostominae Pilsbry & Vanatta, 1898

Plagiodontes Doering, 1877
Type species: *Helix dentata* W. Wood, 1828, from Montevideo, Uruguay [subsequent designation by Pilsbry, 1898].

Plagiodontes strobelii (Doering, 1877)

Bulimus (Odontostomus) daedaleus var. major Strobel, 1874: 16–17 [subspecific name; junior homonym of *B. daedaleus major* Pfeiffer,

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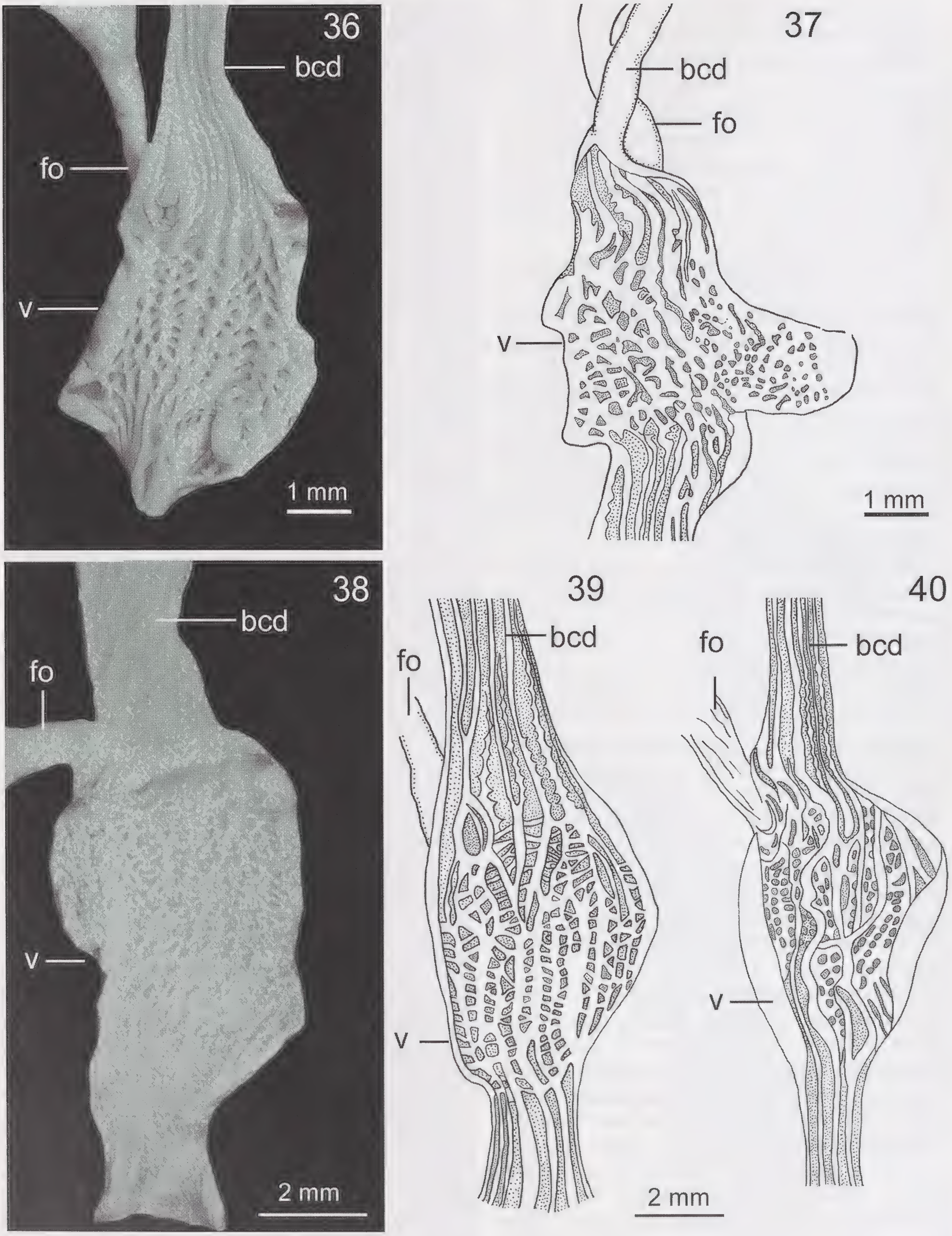
FIGS. 32–35. Terminal male genitalia of *Plagiodontes daedaleus strobelii*. FIGS. 32, 33: Internal structure of penis; FIGS. 34, 35: Internal structure of epiphallus and penial papilla. Abbreviations: e, epiphallus; el, epiphallic lamellae; f, flagellum, hp, hollow papilla of the epiphallus; pi, pilaster; pp, penial papilla; prm, penis retractor muscle; vd, vas deferens.



FIGS. 36–40. Terminal female genitalia of *Plagiodontes daedaleus daedaleus* and *P. daedaleus strobelii*. FIGS. 36, 37: Vaginal internal structure of *P. daedaleus daedaleus*; FIGS. 38–40: Vaginal internal structure of *P. daedaleus strobelli*. Abbreviations: bcd, bursa copulatrix duct; fo, free oviduct; v, vagina.

1853]; Pilsbry, 1901: 98–99 [partim].

Bulimus (Plagiodontes) daedaleus var. strobelii Doering, 1877: 239–240 [replacement name for *var. major* Strobel, 1874; subspecific name]; Doering, 1878: 320–321; Kobelt, 1878: 133.

Bulimus (Plagiodontes) daedaleus var. salinicola Doering, 1877: 240 [subspecific name]; Doering, 1878: 321.

Odontostomus (Plagiodontes) daedaleus var. salinicola – Kobelt, 1878: 133; Pilsbry, 1901: 99.

Cyclodontina (Plagiodontes) daedalea major – Parodiz, 1939: 721 [partim]; 1957: 28 [partim].

Cyclodontina (Plagiodontes) daedalea minor – Parodiz, 1939: 721 [partim]; 1957: 28 [partim].

Scalarinella (Plagiodontes) daedaleus strobelii – Zilch, 1971: 199.

Bulimus (Odontostomus) daedaleus major – Breure, 1974: 118 (catalogue) [partim].

Plagiodontes daedaleus major – Fernández, 1973: 147 [partim]; Richardson, 1993: 54 [partim].

Diagnosis: Elongate-ovate shell, with a conic spire and an acute apex; protoconch and teleoconch sculptures not sharply limited. Penis subcylindrical; penial papilla quadrangular elongated, simple, with no accessory lobe. Vagina subcylindrical, centrally swollen. A comparison to *P. daedaleus daedaleus* is summarized in Table 3.

Type Series: Lectotype, SMF 25351 (Fig. 41) from "Sierra de Aconjigasta, Argentina", as designed by Zilch (1971).

Type locality: Sierra de Aconjigasta [Sierra de Pocho], Córdoba Province, Argentina.

Distribution: Central and northern San Luis province, west slope of Sierra Grande and Sierra de Comechingones, Córdoba province, Argentina.

Status of *Plagiodontes daedaleus salinicola* (Doering, 1877)

Forty one living specimens were found near the type locality of this nominal subspecies ("salty margins of the Laguna de Pocho"; Doering, 1877: 240; 1878: 321), at Provincial Road 28, close to Las Palmas (about 6 km from Laguna de Pocho). Their shells were not different to the 20 specimens collected at Chancaní (29 km far from the type locality).

As described by Doering (1877: 200; 1878: 321), some of these adult shells were smaller than typical *P. strobelii* (range: from 21.70 to 30.05 mm), and somehow similar to *P. daedaleus,* so five shells scored in the overlapping area of the MDA histogram (8.19% of incorrect classifications, that is, fitting the general model of variation found for the whole data set).

Eight additional dissections were made on snails from Las Palmas, confirming that all specimens had a simple penis papilla and a non-swollen vagina that warrant *salinicola* not being a member of *P. daedaleus* but rather a population of *P. strobelii* that does not deserve any subspecific status.

DISCUSSION

A subspecies is "an aggregate of local populations of a species inhabiting a geographical subdivision of the range of the species and differing taxonomically from other populations of the species" (Mayr & Ashlock, 1991). However, formal tests of morphometric discrimination and degree of allopatry were seldom performed for most available trinomina.

Six nominal subspecific names were recorded for *Plagiodontes daedaleus*:

(1) *Plagiodontes daedaleus daedaleus* (Deshayes, 1851): The locality "Brazil" given by Deshayes (1851, in: Férussac & Deshayes, 1851) was erroneous (Pilsbry, 1901; Parodiz, 1939). This form is very common in the Sierras de Córdoba, central Argentina. Doering (1875) described *P. daedaleus minor,* which is a junior synonym of the typical *P. daedaleus daedaleus* (Pilsbry, 1901; Parodiz, 1939).

(2) *Plagiodontes daedaleus major* (Pfeiffer, 1853: 370): It was characterized as a larger, more ventrose variety of *P. daedaleus* ("β. Major, ventrosior") with no geographical restriction. Richardson (1993: 54) recognized Pfeiffer (1853) as the author of *Plagiodontes daedaleus major* as an available name.

(3) *Plagiodontes daedaleus strobelii* (Doering, 1877): Strobel (1874: 16–17) misidentified the largest variety, and described a var. *major* as a shell with a produced, acute

conic spire, from Cerro del Morro, San Luis province, that is, a slender shell, less ventrose than any *daedaleus* form, and with a considerably different spire shape. The name was explicitly intended to be subspecific, since in the same work the author stated that varieties were equivalent to subspecies ("sottospecie" in the Italian original text), or the entities that Darwin called *"specie incipienti"* (Strobel, 1874: 15), and employed the term *mutationes* to describe some infrasubspecific variations.

Doering (1877: 239; 1878: 320) acknowledged that the name *daedaleus* included two main configurations separated by the Sierra de Córdoba*:* the snails on the east slope have ventrose shells of variable size, with a short, blunt spire, like typical *daedaleus*; the snails over the west slope have more elongated, less ventrose shells, with a longer conic spire (i.e., *B. daedaleus major* Strobel, 1874, non Pfeiffer, 1853). Because of this difference, Doering was reluctant to keep Strobel's variety in the same species, and unambiguously stated that he did so only in a provisional way ("provisoriamente la reunimos con el *P. daedalens*" [sic; lapsus calami]: Doering, 1877: 239; 1878: 320). To avoid homonymy, he explicitly replaced the name *major* Strobel, 1874, by *strobelii.*

(4) *Plagiodontes daedaleus multidentatus* (Doering, 1875): Described as similar to *P. daedaleus daedaleus* but with a stronger dentition in the shell aperture. Parodiz (1939) considered it to be a junior synonym of *P. daedaleus daedaleus* (= *P. daedaleus minor*).

(5) *Plagiodontes daedaleus salinicola* (Doering, 1877): It was described as smaller and less slender than *P. daedaleus strobelii,* from Laguna de Pocho in San Luis province.

(6) *Plagiodontes daedaleus costatus* Hylton-Scott, 1952: It was characterized as obese shells, with a blunt apex, like typical *daedaleus,* but with strong axial ribs in the teleoconch. The only documented locality is Copacabana, Córdoba province.

Four out of these six subspecies have obese shells, a blunt apex, a well-defined boundary between the protoconch and the teleoconch, and live to the east of the Pampean Sierras: *P. d. daedaleus, P. d. major, P. d. multidentatus,* and *P. d. costatus.* The remaining two subspecies, *P. d. strobelii* and *P. d. salinicola,* have slender shells with a conical acute apex and live to the west of the sierras.

Our results confirmed the existence of significant morphological gaps between the two groups. Pilsbry (1901) and Parodiz (1939) failed to recognize the shell differences between the two largest varieties, included the misleading statement that *major* Strobel was apparently identical to *major* Doering, and listed the objective synonyms *major* Strobel and *strobelii* Doering as if they had been proposed for different entities. This opinion is rejected here because *P. strobelii* is recognizable as a significantly different morph.

A morphometric discrimination that correctly classifies more than 90% of the shells collected to the east or the west of the sierras should have been a good argument for subspecific recognition, but the existence of definite differences in the genital anatomy between the two groups (especially the presence of either a simple penial papilla or a papilla with an additional lobe) supports the specific status of both forms.

The internal anatomy of *Plagiodontes daedaleus* was still poorly known. Only Breure & Schouten (1985) outlined the genital anatomy of some specimens from Cerro del Morro, San Luis (i.e., the locality mentioned by Strobel, 1874, for his *P. daedaleus major;* now: *P. strobelii*), and Alta Gracia, Córdoba (i.e., from an area within the range of *P. daedaleus*), and Tillier (1989) sketched the pallial complex of a specimen from Cerro del Morro, San Luis, indicating the position of the ureteric pore and the open condition of the secondary ureter.

In this study, all specimens from the east slope of the Pampean Sierras (i.e., *P. daedaleus*) had a papilla with an accessory lobe. By contrast, all specimens from the west slope of the sierras (i.e., *P. strobelii,* including *salinicola*) exhibited a simple penial papilla.

Figure 9 in Breure & Schouten (1985: 18) illustrated the genital system of a specimen from Cerro del Morro. It was labelled as *Plagiodontes dealdalea* [lapsus calami for *daedaleus*], but it shows a subcylindrical penis and a non-swollen vagina, which according to our results allow inferring that the specimen had a simple penial papilla and actually was *P. strobelii*. Conversely, the drawing for *Plagiodontes* sp. from Alta Gracia, Córdoba, in figure 12 of Breure & Schouten (1985: 21) shows a club-shaped penis and a centrally swollen vagina that correspond to typical *P. daedaleus,* as a consequence of having an accessory lobe to the penial papilla.

Parodiz (1939) and Fernández (1973) supplied long lists of localities in Argentina for *P. daedaleus* that included information on the distribution of the overlooked *P. strobelii.* Our listing of studied material (Appendix 1) supports the hypothesis that *P. strobelii* is restricted to western Córdoba and San Luis province, whereas *P. daedaleus* is widely distributed in eastern Córdoba; they are separated by the central sierras and they would not have any degree of sympatry.

Mountain grasslands in the summits seem to be an effective barrier that prevents the expansion of these species towards the opposite side of the sierras. It is suggestive that also *P. patagonicus* appears to be similarly limited, since it only extends southwards, from the summits of the sierras in southern Buenos Aires province to the plains, and was never reported from localities over the northern slope (Cazzaniga et al., 2005).

The presence of either a simple penial papilla or a papilla with an additional lobe, which is correlated with a characteristic widening of penis and vagina, was recently argued by Pizá & Cazzaniga (2009) as a reliable character for distinguishing some species in genus *Plagiodontes,* which is currently composed by nine species.

Other than in *P. strobelii,* a simple penis papilla is only found in *P. dentatus* from Uruguay and eastern Argentina, and *P. patagonicus* from southern Buenos Aires, but its shape is different in these three species: it is short and round in *P. dentatus;* it is proximally swollen and distally triangular and elongated in *P. patagonicus* (Pizá & Cazzaniga, 2009), and it is quadrangular elongated in *P. strobelii.*

All the remaining species in the genus have an accessory lobe that derives in a club-shaped penis. The accessory lobe was described by Pizá & Cazzaniga (2009) as smaller than the penial papilla in *P. multiplicatus parvus* Hylton-Scott, 1952, and *Plagiodontes weyrauchi* Pizá & Cazzaniga, 2009. *Plagiodontes daedaleus* also has a papilla larger than the accessory lobe (this paper). The penial papilla of *P. brackebuschii* and *P. weyenberghii* also has a small accessory lobe, which is similar to that of *P. daedaleus* (Pizá, MS). Also, *P. rocae* Doering, 1881, has an accessory lobe but in this species the lobe is larger than the papilla itself.

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APPENDIX 1

Studied collections of *Plagiodontes daedaleus* and *P. strobelii*. S: species (D: *P. daedaleus;* S: *P. strobelii*); M: sets included in morphometric analyses of shells; A: sets on which pallial and genital anatomy was studied. Altitude in m a.s.l. Province abbreviations: C, Córdoba; SL, San Luis.

Set	S	M	A	Locality (Province): Lat., Long.; Altitude (m a.s.l.)	Collection data; collector [previous museum label]; Note
1	*P. d.*	X	X	Embalse−Berrotarán (C): 32°20'00.6"S, 64°21'41.9"W; 628	Own material; J. Pizá & M. Carrizo, 2005.
2	*P. d.*	X		Embalse (C): 32°14'29.1"S, 64°21'43"W; 630	FML 1277/6; W. Weyrauch [*P. daedaleus*].
3	*P. d.*	X	X	Alta Gracia (C): 31°39'01.5"S, 64°27'56.6"W; 651	Own material; J. Pizá & M. Carrizo, 2005.
4	*P. d.*			La Granja. Alta Gracia (C): ~31°40'S, ~64°26'W	MACN-In 14204; Bruch.
5	*P. d.*	X	X	Observatorio (C): 31°35'46.5"S, 64°32'39.4"W; 1,455	Own material; J. Pizá & M. Carrizo, 2005.
6	*P. d.*	X		Carlos Paz (C): ~31°25'S, ~64°28'W	MACN-In 26589; A. Castellanos.
7	*P. d.*	X		Tanti (C): 31°21'33.9"S, 64°35'17.3"W; 921	Own material; J. Pizá & M. Carrizo, 2005.

(continues)

(continued)

Set	S	M	A	Locality (Province): Lat., Long.; Altitude (m a.s.l.)	Collection data; collector [previous museum label]; Note
8	*P. d.*	X		Pan de Azúcar (Cosquín) (C): ~31°14’S, ~64°25’W; 800	Own material; Pablo Martín.
9	*P. d.*			Pampa de Olaen (C) : ~31°10’S, ~64°36’W; ~1,120	MLP 9736.
10	*P. d.*	X	X	Valle Hermoso (C): 31°06’49.5”S, 64°28’09.3”W; 999	Own material; J. Pizá & M. Carrizo, 2005.
11	*P. d.*			La Falda (C): ~31°05’S, ~64°30’W	MACN-In 23555; Haedo [*P. daedaleus major*].
12	*P. d.*	X		Huerta Grande (C): ~31°04’S, ~64°30’W; 963	Own material; Pablo Martín.
13	*P. d.*	X	X	Jesús María (C): 30°58’58.6”S, 64°06’43.1”W; 531	Own material; J. Pizá & M. Carrizo, 2005.
14	*P. d.*	X		La Cumbre (C): 30°58’36.6”S, 64°28’52.6”W; 1,221	Own material; Pablo Martín.
15	*P. d.*	X	X	Los Cocos−La Cumbre (C): 30°57’16.4”S, 64°31’23”W; 1,107	Own material; J. Pizá & M. Carrizo, 2005.
16	*P. d.*	X		San Esteban (C): ~30°55’S, ~64°32’60”W; 1,007	MLP no number; M. I. Hylton-Scott.
17	*P. d.*	X		Dolores (C): ~30°54’S, ~64°32’W; 1,026	MLP 36015.
18	*P. d.*	X	X	Los Cocos−Capilla del Monte (C): 30°53’37.6”S, 64°32’01.7”W; 1,014	Own material; J. Pizá & M. Carrizo, 2005.
19	*P. d.*			Soto (C), ~30°51’S, ~65°01’W	MLP 6693 [*Plagiodontes daedaleus*].
20	*P. d.*	X	X	Ongamira (C): 30°46’08.1”S, 64°29’07.8”W; 1,076	Own material; J. Pizá & M. Carrizo, 2005.
21	*P. d.*	X	X	Ischilín (C): 30°36’06.336”S, 64°20’52.908”W; 913	Own material; J. Pizá & M. Carrizo, 2007.
22	*P. d.*	X	X	Deán Funes−Ischilín (C): 30°29’24.9”S, 64°22’19.2362”W; 822	Own material; J. Pizá & M. Carrizo, 2007.
23	*P. d.*	X		Avellaneda (C): 30°34’S, 64°12’W; 675	FML 24; W. Weyrauch, 1969 [*Plagiodontes daedaleus*].
24	*P. d.*	X		Villa Totoral (C): 30°40’58.1”S, 64°00’59.9”W; 523	Own material; J. Pizá & M. Carrizo, 2005.
25	*P. s.*			Cerro del Morro (SL): ~33°08’S, ~65°26’W	MACN-In 30680; F. Pastore [*P. daedaleus major*].
26	*P. s.*	X		Cerro Rosario (SL): 32°57’0”S, 65°42’14.8”W; 1,110	FML 1304; W. Weyrauch [*P. brakkebuschii attenuatus* Weyrauch (*nomen nudum*)].
27	*P. s.*			To the West of Renca (SL): ~32°45’S, ~65°22’W	MACN-In 16226; A. Castellanos [*Odontostomus*].
28	*P. s.*			San Francisco del Monte de Oro (SL), Quebrada del río Gomez: 32°37’02”S, 66°04’17.3”W; 805	Own material; J. Pizá & M. Carrizo, 2009.
29	*P. s.*	X	X	San Francisco del Monte de Oro (SL): 32°37’30”S, 66°08’49.5”W; 896	Own material; J. Pizá & M. Carrizo, 2005.
30	*P. s.*			Sierra de San Francisco (SL): ~32°37’S, ~66°08’W	MACN-In 15130; A. Castellanos y Serié [*Odontostomus (Plagiodontes) daedaleus* var. *major*].

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Set	S	M	A	Locality (Province): Lat., Long.; Altitude (m a.s.l.)	Collection data; collector [previous museum label]; Note
31	*P. s.*			San Francisco del Monte de Oro (SL): ~32°37'S, 66°08'W	MACN-In 16750; [*Odontostomus* sp.].
32	*P. s.*			Dique La Huertita (SL): 32°24'11.4"S, 65°43'28.5"W; 1,004	Own material; J. Pizá & M. Carrizo, 2009.
33	*P. s.*		X	Provincial Road # 2, San Martín-Quines (SL): 32°19'32.8"S, 65°41'39.3"W; 808	Own material; J. Pizá & M. Carrizo, 2009.
34	*P. s.*			Sierras de Luján (SL): ~32°22'S, ~65°57'W	MACN-In 15135; A. Castellanos y Serié [*Odontostomus (Plagiodontes) daedaleus major*].
35	*P. s.*			Sierras de Quines (SL): ~32°13'S, ~65°47'W	MACN-In 15126; A. Castellanos y Serié [*Odontostomus (Plagiodontes) daedaleus* var. *major*].
36	*P. s.*			La Quebrada, Santa Rosa–Quines (SL)	MACN-In 1639; C. Vega [*Odontostomus (Plagiodontes) daedaleus major*].
37	*P. s.*	X		Quebrada de Cautana (SL): ~32°17'S, ~65°30'W; ~600	MACN-In 31616; R. Bidart [*Plagiodontes* sp.].
38	*P. s.*			Quebrada de Cautana (SL): 32°12'45.3"S, 65°21'37.3"W; 683	Own material; J. Pizá & M. Carrizo, 2009.
39	*P. s.*	X		Bajo de Veliz (SL): ~32°19'S, ~65°20'W; ~600	MLP no number; A. Castellanos [*P. daedaleus mayor* (Strobel)].
40	*P. s.*			Bajo de Veliz (SL): 32°19'18.5"S, 65°24'28.5"O; 659	Own material; J. Pizá & M. Carrizo, 2009.
41	*P. s.*	X		Santa Rosa de Conlara (SL): 32°19'55.8"S, 65°14'51.9"W; 619	Own material; J. Pizá & M. Carrizo, 2005.
42	*P. s.*			Rincón. Depto. Junín (SL): ~32°26'S, ~65°28'W	MACN-In 18313; Yepes [*Odontostomus* sp.].
43	*P. s.*			Cortaderas, near Rodríguez Saa station (SL): ~32°30'S, ~65°12'W	MACN-In 23550; C. Vega 1938 [*Odontostomus (Plagiodontes) daedaleus major*].
44	*P. s.*			San Javier (C): ~32°02'S, ~65°03'W	MACN-In 1653; A. Castellanos.
45	*P. s.*	X		Dique La Viña (C): ~31°53'S, ~65°01'W; 885	MACN-In 3241; J. Jurado [*Plagiodontes* sp.].
46	*P. s.*			Dique La Viña (C): ~31°53'S, ~65°01'W; 885	MACN-In no number; Gancedo [*P. brackebuschi*].
47	*P. s.*	X		Dique La Viña (C): 31°53'19"S, 65°01'50"W; 885	Own material; J. Pizá & M. Carrizo, 2005.
48	*P. s.*			Mina Clavero (C): ~31°42'S, ~65°00'W	MACN-In 9245; C. de Villalobos [*Odontostomus*].
49	*P. s.*	X	X	Chancaní Provincial Park (C): 31°20'12.72"S, 65°29'5.82"W; 330	Own material; S. Burela & N. Pelegrin.
50	*P. s.*	X	X	Provincial Road # 28 near Las Palmas (C): 31°21'49.3"S, 65°12'17.3"W; 1,083	MACN-In 37086; J. Pizá & M. Carrizo, 2005 [*P. daedaleus*].
51	*P. s.*	X		Taninga–Salsacate (C): ~31°12'S, ~65°08'W; 840	FML 914; W. Weyrauch [*P. daedaleus*].

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MOLECULAR PHYLOGENETIC REVISION OF THE FRESHWATER LIMPET GENUS *FERRISSIA* (PLANORBIDAE: ANCYLINAE) IN NORTH AMERICA YIELDS TWO SPECIES: *FERRISSIA* (*FERRISSIA*) *RIVULARIS* AND *FERRISSIA* (*KINCAIDILLA*) *FRAGILIS*

Andrea C. Walther, John B. Burch & Diarmaid Ó Foighil*

Museum of Zoology and Department of Ecology and Evolutionary Biology,
University of Michigan, Ann Arbor, Michigan 48109-1079, U.S.A.

ABSTRACT

Members of the gastropod genus *Ferrissia* Walker, 1903, have a near-cosmopolitan distribution in freshwater ecosystems. Five North American species, distinguished by conchological, habitat, and distributional details, are generally recognized. However, they can be difficult to diagnose in practice, and we aimed to comprehensively revisit their systematics and taxonomy using molecular phylogenies. Our primary result was congruent for all three genetic markers: two highly distinctive *Ferrissia* clades, each encompassing multiple nominal species, were widely distributed across North American watersheds in both lentic and in lotic habitats. One clade was restricted to North America and was exclusively composed of lotic *F. rivularis* (Say, 1817) and lentic *F. parallela* (Haldemann, 1841). These two taxa exhibited geographic, rather than taxonomic, genetic structuring, strongly implying that they are conspecific and that *F. parallela* is a junior synonym of *F.* (*Ferrissia*) *rivularis*. The other clade contained three North American taxa, *F. fragilis* (Tryon, 1863) and putative specimens of *F. mcneilli* Walker, 1925, and *F. walkeri* (Pilsbry & Ferriss, 1907), in addition to the Hawaiian *F. sharpi* (Sykes, 1900), as well as multiple invasive Eurasian founder populations. All four taxa shared overlapping genotypes and shell phenotypes, prompting us to conclude that they are conspecific with *F. fragilis* having taxonomic priority. This New World species had robust phylogenetic links to Old World congeners, thereby enabling us to unite global septum-forming *Ferrissia* species under the subgenus *Kincaidilla* Hannibal, 1912. *Ferrissia rivularis* and *F. fragilis* differed in a number of conchological traits, including maximum body size, details of apex positioning and orientation, and the ability to form a septum. The two former traits were sufficient to distinguish sympatric populations.

Key words: biodiversity, gastropod, freshwater, limpet, phylogeny, taxonomy.

INTRODUCTION

North American freshwater limpets have been relatively understudied since Paul Basch's classic body of work (Basch, 1959, 1960, 1962a, b, 1963a, b). Taxonomic uncertainty among ancylinid limpets, by far the most widespread and common constituent group, has contributed to this neglect. It pervades the literature and it is quite common for ancylinid study taxa to be identified to generic (Morgan et al., 2002) or familial/subfamilial (Stewart et al., 1998; McMahon, 2004) status only. This unsatisfactory situation stems in part from inadequate taxonomic descriptions (Basch, 1963a; Jokinen, 1978; McMahon, 2004) – see Burch (2009) for a detailed compilation of original species descriptions – exacerbated by pronounced conchological plasticity (Basch, 1963a, b; Russell-Hunter et al., 1981; McMahon, 2004; Hovingh, 2004; Dillon & Herman, 2009).

A number of recent molecular phylogenetic studies have helped put ancylinid systematics and taxonomy on a firmer footing, for example, excluding convergent freshwater limpet lineages (Albrecht et al., 2004), confirming the linkage with planorbids (Morgan et al., 2002; Albrecht et al., 2004; Walther et al., 2006a), and detecting basal New World and Holarctic sister clades (Walther, 2006a). The phylogenetic placement of ancylinids within Planorbidae, in association with earlier anatomical work (Hubendick, 1978),

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*Corresponding author: diarmaid@umich.edu

TABLE 1. Morphology and habitat of the five nominal species of North American *Ferrissia* recognized by Basch (1963).

Nominal species	Shell characteristics	Habitat & Distribution	Type Locality
F. rivularis (Say, 1817)	Robust, ≤ 7 mm, elevated, elliptical aperture, apex in midline or slightly to the right.	Rivers and streams across the U.S.	Unknown rivulets
F. parallela (Haldeman, 1841)	Thin-shelled, large, ≤ 9 mm, elevated and very narrow, peritreme often arched, obtuse apex in midline.	Lakes, northern states and Canada	New England
F. fragilis (Tryon, 1863)	Fragile & minute, ≤ 4 mm, moderately elevated or depressed, oval aperture, can form a septum, sub-acute or obtuse apex in right-posterior quadrant.	Small bodies of standing water, often temporary, across the U.S.	Laguna Honda, California
F. walkeri (Pilsbry & Ferriss, 1907)	Thin-shelled, ≤ 6 mm, usually depressed, oval aperture, apex subacute, often far into the posterior-right quadrant.	Ponds and lakes, Arkansas, Michigan, southern California	Leafy fish pond in Rogers, Benton Co., Arkansas
F. mcneilli Walker, 1925	Fragile, ≤ 5 mm, very much depressed, often a glossy red-brown color, apex a rounded bump in right-posterior quadrant.	Streams, Mobile Bay drainage, Alabama	Mandeville Creek, Mobile Co., Alabama

has prompted the taxonomic revision of these limpets from familial (Ancylidae) to subfamilial (Ancylinae) status (Albrecht et al., 2007; Strong et al., 2008). Molecular phylogenies have also proven useful for ancylinid biodiversity studies, for example, taxonomic revision of the genus *Laevapex* (Walther et al., 2006a) and detection of cryptic endemic (Pfenninger et al., 2003; Albrecht, 2006) and invasive (Walther et al., 2006b, c) species.

The ancylinid genus *Ferrissia* Walker, 1903, has been recorded from lentic and lotic freshwater habitats across North America and on every continent except Antarctica (Basch, 1963a; Hubendick, 1964; Clarke, 1973; Gómez et al., 2004; Thiengo et al., 2004). The genus is characterized by an apical microsculpture of fine radiating grooves formed during embryogenesis (Walker, 1903; Basch, 1963a; Hubendick, 1964), a flagellum-bearing simple penis (Hoff, 1940; Basch, 1963a; Hubendick, 1964), and a single-lobed pseudobranch that bears the anus (Hoff, 1940; Basch, 1963a). Hubendick (1964) established New World and Old World subgenera (respectively *Ferrissia* and *Pettancylus*) based on details of penial complex morphologies.

Basch (1963a) synonymized over 20 nominal North American *Ferrissia* taxa into five species (*F. rivularis*, *F. parallela*, *F. fragilis*, *F. walkeri*, and *F. mcneilli*) distinguished by details of their shell morphologies and their habitats and distributions (Table 1). He was very aware of ecophenotypic conchological variation and the potential difficulty this posed for diagnoses based on fine-scale conchological features (Basch, 1963b). Consequently, habitat fidelities (lotic for *F. rivularis* and *F. mcneilli*; lentic for *F. fragilis*, *F. parallela* and *F. walkeri*) are critical attributes of his taxonomic scheme. However, Basch (1963a) was open to the possibility of evolutionary transition among habitats, speculating that the lentic taxa *F. parallela* and *F. walkeri* may have evolved from an ancestral lotic *F. rivularis* lineage. With a few minor exceptions, for example, *F. fragilis* synonyms (Thompson, 1999), Basch's (1963a) taxonomy of North American *Ferrissia* has generally remained widely accepted (Clarke, 1973; Jokinen, 1978; Hovingh, 2004; Fritz & Feminella, 2006; Pyron et al., 2008). Nevertheless, multiple authors report inconsistencies, including the occurrence of species morphotypes in ostensibly ectopic habitats – e.g., lotic *F. walkeri* (Hovingh, 2004) and *F. fragilis* (Pyron et al., 2008 – and the difficulty of distinguishing *F. fragilis* and *F. walkeri* (Jokinen, 1978), a shortcoming also acknowledged by Basch (1963a).

A small number of *Ferrissia fragilis*, *F. rivularis* and *F. parallela* have been included in previous molecular phylogenetic studies (Walther et al., 2006a, b, c). All three taxa placed in a robust ancylinid clade comprising the genera *Ferrissia*, *Rhodacmea*, and *Ancylus*. However, the three congeners were not monophyletic: *F. rivularis* and *F. parallela* exhibited a very close phylogenetic relationship, but *F. fragilis* was topologically disjunct, being either weakly sister to *Rhodacmea* and *Ancylus* or forming part of a basal ancylinid polytomy (Walther et al., 2006a, b). *Ferrissia fragilis* appears to lack North American sister taxa and has complex phylogenetic ties to Old World *Ferrissia* populations, including an apparent sister relationship with African *F.* cf. *clessiniana* (Jickeli, 1882) (Walther et al., 2006b), as well as cryptic, and frequently misidentified, invasive founder populations in Europe and Asia (Walther et al., 2006b). In contrast with the above studies, Dillon & Herman (2009) recently proposed synonymization of *F. rivularis* and *F. fragilis* based on allozymic and morphometric analyses of two South Carolina lotic populations, one from a fast flowing river, the other a languid creek. They reached this conclusion despite acknowledging that exemplar specimens forwarded to us for genotyping from both of their study populations placed unambiguously within the *F. fragilis* clade (Dillon & Herman, 2009).

Our goal in this study was to revisit North American *Ferrissia* taxonomy using molecular phylogenetic datasets generated from an extensive sampling of continental populations. Our data indicate that Basch's (1963a) taxonomic scheme requires further consolidation: only two species, *F. rivularis* and *F. fragilis*, appear to be phylogenetically supportable. Both taxa are widespread in continental lentic and lotic habitats, and we provide guidelines for distinguishing them morphologically.

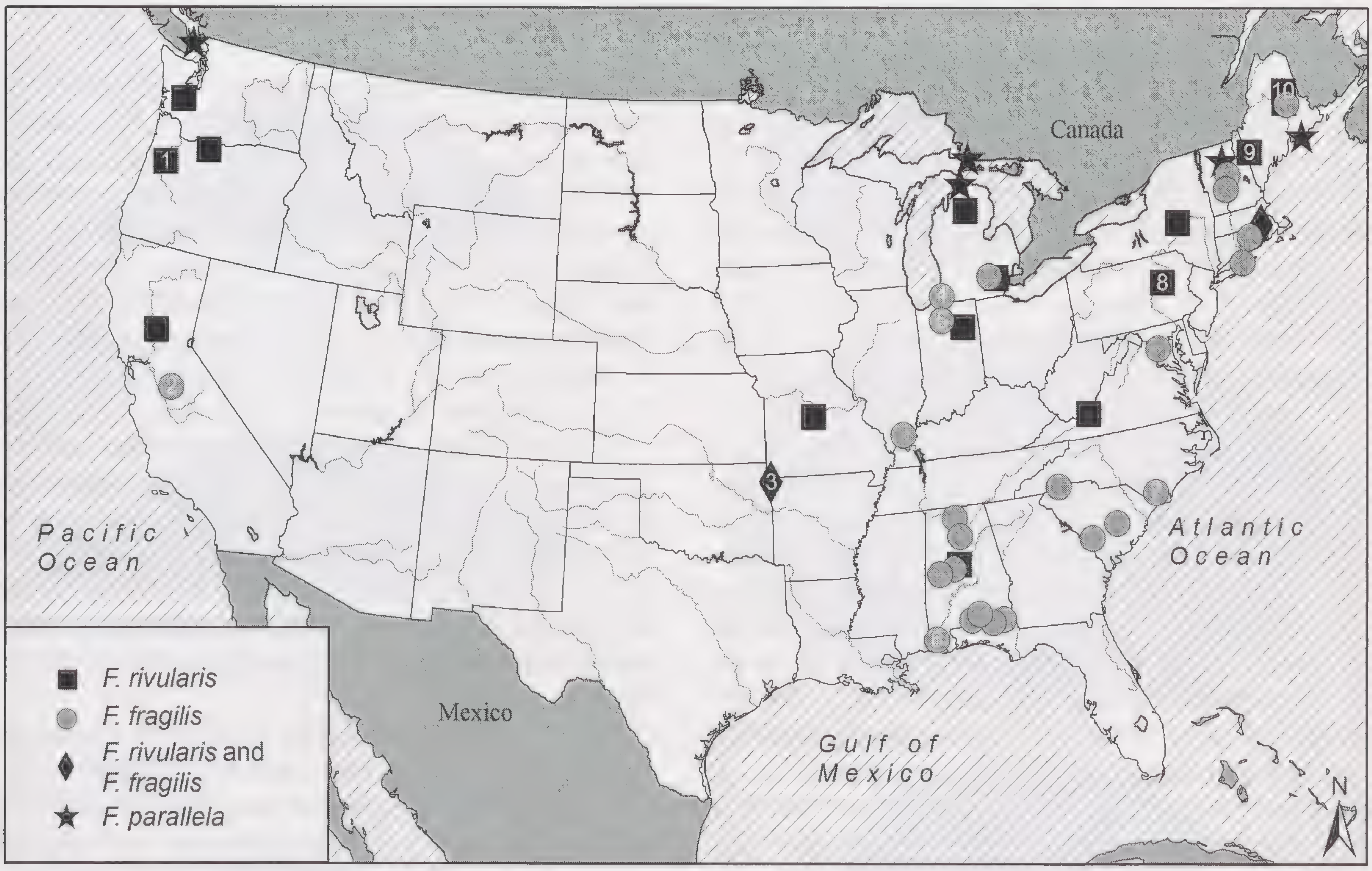

FIG. 1. Map showing the approximate locations of our North American *Ferrissia* species sampling localities; Table 2 has detailed locality information. Samples were identified using Basch's (1963a) morphological criteria, but note that we were unable to unambiguously identify specimens of *Ferrissia walkeri* or *F. mcneilli*. Sampling sites at, or near, type localities are identified numerically: 1 = *Ancylus oregonensis* (syn. *F. rivularis*), 2 = *Gundlachia californica* (syn. *F. fragilis*), 3 = *A. walkeri* (syn. *F. walkeri*), 4 = *F. michiganensis* (syn. *F. walkeri*), 5 = *F. bartschi* (syn. *F. fragilis*), 6 = *F. mcneilli*, 7 = *Ancylus* (*F.*) *hendersoni* (syn. *F. fragilis*), 8 = presumed *F. rivularis* type locality, 9 = *Ancylus ovalis* (syn. *F. rivularis*), 10 = *Ancylus borealis* (syn. *F. rivularis*).

MATERIALS AND METHODS

Specimen Collection

Ferrissia populations were sampled from a diversity of lentic and lotic habitats throughout the eastern U.S. as well as from Pacific Coast drainages from California to British Columbia (Fig. 1; Table 2). Our initial aim to collect from topotypic populations was compromised by either an absence of meaningful type locality information (*F. rivularis* and *F. parallela*; Table 1), or vague locality information coupled with extensive subsequent landscape changes that rendered it impossible to confidently resample the actual type localities (*F. fragilis*, *F. mcneilli* and *F. walkeri*; Table 1), We ameliorated these shortcomings by obtaining samples from: (1) populations as close to type localities as possible (e.g., Benton County, Arkansas, ponds for *F. walkeri*); (2) still tractable sites where the target taxon had been reliably sampled, e.g., Eslava Creek, Alabama, the source of UMMZ 161631 *F. mcneilli* sampled by McNeill and identified by Walker; (3) type localities of *F. fragilis*, *F. rivularis* and *F. walkeri* synonyms (Fig. 1; Table 2). We also incorporated new extra-continental samples of *Ferrissia* (the putative endemic *F. sharpi* from Hawaii and suspected invasive *F. fragilis* from the Azores and France), as well as a North American ancylinid, *Hebetancylus excentricus* that had been unavailable during our previous studies (Walther et al., 2006a, b, c). All specimens were preserved in 95% ethanol.

Individual limpets were assigned to the genus *Ferrissia* based on their possession of diagnosic radial apical microsculpture (Basch, 1963a; Burch, 1988), and species identifications were made based on available keys and descriptions (Basch, 1963a; Table 1), in addition to comparison with holotype material (Fig. 2). Note, however, that the *F. parallela* holotype is much smaller than the maximum adult size (9 mm in length; Basch, 1963a) for this species, and the *F. fragilis* holotype is partially damaged (Fig. 2). Basch (1963a) emphasized that his *Ferrissia* key applied to adult specimens only and that sub-adults might well prove intractable. We concur: his key readily identified larger specimens of *F. rivularis* in rivers, *F. parallela* from northern ponds and lakes and *F. fragilis* from ponds and lakes throughout the U.S. Small specimens proved more difficult, and many of the limpets < 4 mm in length sampled from the slower-moving sections of creeks and rivers resembled *F. fragilis*. We had significant problems identifying unambiguous members of the remaining two species. Basch's (1963a) description of *F. mcneilli* is perfunctory and includes no diagnostic characters to distinguish it from *F. fragilis* except habitat: respectively Mobile, Alabama, area streams versus ponds/ditches continent-wide. Our Mobile, Alabama, stream samples matched Basch's (1963a) description of *F. fragilis*. Basch (1963a) differentiated *F. walkeri* from *F. fragilis* by the former's larger size and habitat of larger bodies of standing water – however, the former's type locality is a pond (Table 1) – but we concur with Jokinen (1978) that the species descriptions overlap and that it is very difficult to confidently distinguish these two taxa. Our samples from *F. walkeri* near-topotypic (Benton County, Arkansas) and toposyntypic (Berrien County, Michigan) locations best fit Basch's (1963a) *F. fragilis* description, and none of our extensive sampling of North American *Ferrissia* populations have, to date, recovered unambiguous specimens of *F. walkeri*.

Molecular & Phylogenetic Methods

DNA was extracted and target fragments of three genes – 808 nt (aligned) of nuclear ribosomal large-subunit (28S), 639 nt (aligned) of nuclear ribosomal second internal transcribed spacer (ITS-2) and 671 nt of mitochondrial mt *cytochrome oxidase I* (COI) – were amplified and directly sequenced (both strands) using the molecular techniques and primer pairs detailed in Walther et al. (2006a). Edited sequences for each gene fragment were aligned using ClustalW (Thompson et al., 1994), implemented in Sequence Navigator (Applied Biosystems, Foster City, California), and refined manually. Novel genotypes were assembled in a NEXUS format using Sequence Monkey 2.9.0 (available at http://sequence_monkey.tripod.com) and they have been deposited in GenBank (GU391035–GU391217).

PAUP* 4.0b10 (Swofford, 2003) and MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003) were employed for phylogenetic analyses. Maximum parsimony searches were heuristic, with equal character weighting, inferred sequence gaps coded as missing data, 100 random stepwise additions, and tree-bisection-reconnection (TBR) branch-swapping. Bootstrap (Felsenstein, 1985) branch support (BS) levels were estimated with 100 replicates, 100 random additions each. The nearest-neighbor interchange (NNI) branch-swapping algorithm was employed for generating ITS-2 bootstrap support, though

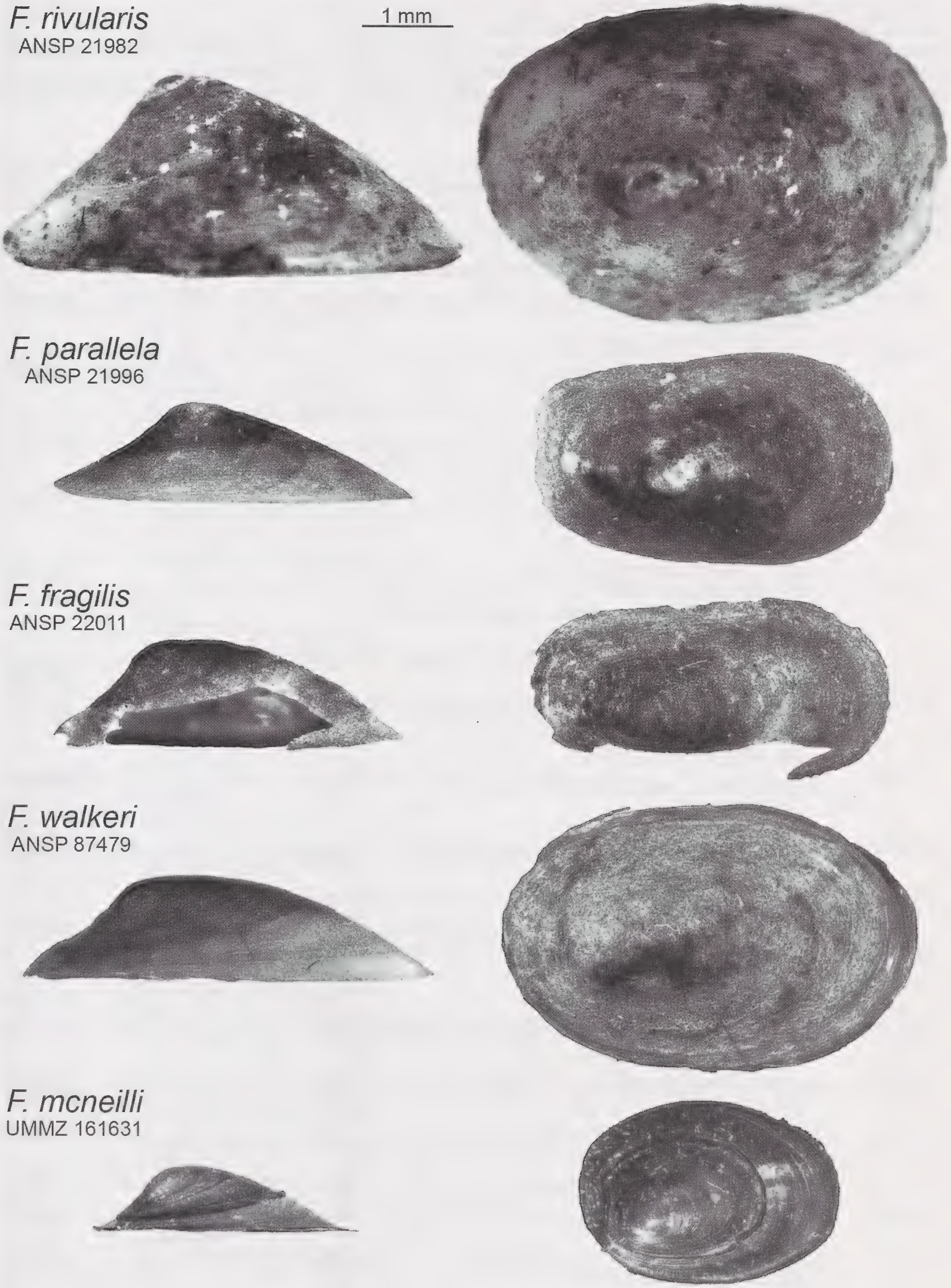

FIG. 2. Lateral and dorsal photographs of museum type and reference shells for the five North American *Ferrissia* species recognized by Basch (1963a). The *F. parallela*, *F. fragilis*, and *F. walkeri* specimens shown are holotypes; the *F. rivularis* specimen is a syntype; and the *F. mcneilli* was collected by L. H. McNeill near the taxon's type locality and identified by Walker.

TABLE 2. Taxonomy, sampling location, location code, genotype information (# individuals sequenced/# unique genotypes obtained), and voucher information (University of Michigan Museum of Zoology [UMMZ] number, unless otherwise noted) for the ancylinid study populations. North American taxa were identified using Basch's (1963a) morphological criteria, but note that we were unable to unambiguously identify specimens of *Ferrissia walkeri* or *F. mcneilli*. Samplings sites at, or near, type localities are indicated in bold.

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
Ferrissia rivularis	Black River, northeast of Littlerock, Thurston Co., Washington [46.9186°N; 123.0011°W]	WAB	-	2/1	1/1	301123
	Black River, just south of Mumby, Thurston Co., Washington [46.8678°N; 123.0178°W]	WAC	1/1	3/2	1/1	301124
	Black Lake Ditch, west of Tumwater, Thurston Co., Washington [47.0017°N; 122.9525°W]	WAD	-	2/1	1/1	301125
	Black River, Littlerock, Thurston Co., Washington [46.9014°N; 123.0172°W]	WAL	-	4/1	1/1	301126
	Small tributary (of Black River?), north of Littlerock, Thurston Co., Washington [46.9186°N; 123.0178°W]	WAS	-	4/3	-	301127
	Battle Creek south of Salem, Marion Co., Oregon [44.8514°N; 123.0517°W] ***near *Ancylus oregonensis* (syn. *F. rivularis*) type locality**	ORB	-	4/2	-	301128
	Mill Creek in The Dalles, Wasco Co., Oregon [45.6006°N; 121.1853°W]	ORL	3/1	12/3	6/2	301204, 301205
	Mill Creek, southwest of The Dalles, Wasco Co., Oregon [45.5678°N; 121.2342°W]	ORM	1/1	5/3	1/1	301129
	Pudding River, east of Salem, Marion Co., Oregon [44.9511°N; 122.8667°W]	ORP	-	4/2	-	301130
	Willamette River, southwest of Salem, Marion Co., Oregon [44.8675°N; 123.1353°W]	ORW	1/1	5/2	1/1	301131
	Feather River at Oroville Wildlife Area, south of Oroville, Butte Co., California [39.4511°N; 121.6022°W]	CAF	1/1	1/1	2/1	301132
	Missouri, USA	MOA	-	-	5/1	301179
	Little River, Huntington, Huntington Co., Indiana [40.8681°N; 85.5011°W]	INL	1/1	1/1	1/1	301178
	AuSable River, Crawford Co., Michigan [44.6648°N; 84.6419°W]	MIA	1/1	1/1	1/1	300224
	Fleming Creek, Washtenaw Co., Michigan [42.3050°N; 83.6617°W]	MIF	-	5/2	-	300280
	Small tributary entering Crystal Lake, Benton Co., Arkansas [36.3352°N; 94.4338°W]	ARL	3/1	-	3/1	301194
	Little Cahaba River, Bibb Co., Alabama [33.0545°N; 86.9703°W]	ALK	1/1	2/1	-	300226
	Oaks Creek, Toddsville, Otsego Co., NY [42.8670°N; 74.9572°W]	NYO	-	2/2	2/1	301134

(continues)

(continued)

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Briar Creek, just west of Berwick, Columbia Co., Pennsylvania [41.0456°N; 76.2851°W]	PAB	1/1	1/1	1/1	301133
	Susquehanna River, Watsontown, Northumberland Co., Pennsylvania [41.0773°N; 76.8587°W] ***presumed *Ancylus rivularis* (syn. *F. rivularis*) type locality, according to Basch(1963a)**	PAS	1/1	1/1	1/1	301135
	New River, Giles Co., Virginia [37.3150°N; 80.6433°W]	VAN	-	1/1	-	300225
	Androscoggin River, West Bethel, Oxford Co., Maine [44.4068°N; 70.8612°W] ***near *Ancylus ovalis* (syn. *F. rivularis*) type locality**	MEA	-	4/2	-	301136
	Fish Stream, Patten, Penobscot Co., Maine [45.9925°N; 68.4413°W]	MEF	-	1/1	1/1	301137
	Rockabema Stream, just southwest of Medway, Penobscot Co., Maine [45.5946°N; 68.5482°W]	MEO	1/1	5/2	1/1	301138
	Rowe Brook, just west of Patten, Penobscot Co., Maine [46.0013°N; 68.4784°W] ***near *Ancylus borealis* (syn. *F. rivularis*) type locality**	MER	1/1	3/2	1/1	301139
	Sweets Pond, Mansfield, Bristol Co., Massachusetts [41.9895°N; 71.2550°W]	MAS	1/1	-	-	301201
F. parallela	Pond on North Pender Island, British Columbia, Canada [48.7358°N; 123.2356°W]	BC	3/1	1/1	1/1	301181
	Douglas Lake, Cheboygan Co., Michigan [45.5797°N; 84.6713°W]	MIL	1/1	1/1	1/1	300229
	Duck Island, Chippewa Co., Michigan [46.3667°N; 84.1347°W]	MID	-	4/1	-	301180
	Joe's Pond, West Danville, Caledonia Co., Vermont [44.4096°N; 72.2019°W]	VTJ	1/1	2/1	1/1	301140
	Kettle Pond, Lanesboro, Caledonia Co., Vermont [44.2964°N; 72.3077°W]	VTK	1/1	4/2	1/1	301141
	Beaver Dam Pond in Acadia National Park, Mt. Desert Island, Hancock Co., Maine [44.3623°N; 68.1956°W]	MEB	1/1	4/1	-	301142
	Hamilton Pond, Mt. Desert Island, Hancock Co., Maine [44.4273°N; 68.2843°W]	MEH	1/1	4/1	1/1	301143
F. fragilis	Edendale Creek, north of Merced, Merced Co., California [37.4189°N; 120.5011°W]	CAE	-	4/2	-	301144

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(continued)

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Merced River at Henderson Park, north of Merced, Merced Co., California [37.5175°N; 120.4172°W] ***near *Gundlachia californica* (syn. *F. fragilis*) type locality**	CAM	1/1	4/2	1/1	301145
	Yosemite Lake, northeast of Merced, Merced Co., California [37.3683°N; 120.4178°W]	CAY	-	5/3	-	301146
	Mexico, Veracruz, Xalapa, Jardin Botanico Clavijero	MXV	1/1	-	1/1	UGSB 1206
	Small creek above Burden Falls at Shawnee National Forest, Illinois [37.5634°N; 88.6422°W]	ILB	-	-	4/1	301182
	Pickerel Lake, Washtenaw Co., Michigan [42.4097°N; 83.9838°W]	MIP	1/1	10/2	2/1	300227, 301174
	Stream west of Harbert, Berrien Co., Michigan [41.8733°N; 86.6371°W] ***at *F. michiganensis* (syn. *F. walkeri*) type locality**	MIW	-	3/1	5/1	301184
	Small tributary entering Crystal Lake, Benton Co., Arkansas [36.3352°N; 94.4338°W] ***near *Ancylus walkeri* (syn. *F. walkeri*) type locality**	ARL	2/1	1/1	1/1	301193
	Lake Maxinkuckee, Culver, Indiana [41.1839°N; 86.3850°W] ****F. bartschi* (syn. *F. fragilis*) type locality**	INM	1/1	-	-	301176
	Big Creek, south of Dothan, Houston Co., Alabama [31.0730°N; 85.4127°W]	ALA	-	2/2	2/1	301154
	Cahaba River at Booth's Ford, Shelby Co., Alabama [33.1853°N; 87.0015°W]	ALB	1/1	2/1	-	300192
	Cahaba River at CR 52 Crossing, Shelby Co., Alabama [33.2842°N; 86.8828°W]	ALC	-	2/2	-	300193
	Cahaba River, Centreville, Bibb Co., Alabama [32.9448°N; 87.1405°W]	ALD	-	1/1	1/1	301155
	Eslava Creek, Mobile Co., Alabama [30.6793°N; 88.1464°W] ***near *F. mcneilli* type locality**	ALE	-	1/1	1/1	301164
	Mouth of Eslava Creek at Eslava Lake, Mobile Co., Alabama [30.6787°N; 88.1529°W] ***near *F. mcneilli* type locality**	ALF	1/1	1/1	1/1	301166
	Big Brush Creek, Wedgeworth, Hale Co., Alabama [32.8172°N; 87.7506°W]	ALG	-	2/1	2/2	301156
	Conecuh River, between Fairfield and Brooklyn, Covington Co., Alabama [31.1856°N; 86.6689°W]	ALH	-	2/1	2/2	301157

(continues)

(continued)

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
	Limestone Creek, Limestone Co., Alabama [34.6269°N; 86.8840°W]	ALL	-	1/1	1/1	301158
	Choctawhatchee River, Eunola, Geneva Co., Alabama [31.0294°N; 85.8538°W]	ALR	-	2/1	2/1	301159
	Yellow River, Covington Co., Alabama [31.3577°N; 86.3421°W]	ALY	-	3/3	2/1	301160
	Mulberry Fork of Black Warrior River, Bangor, Blount Co./Cullman Co., Alabama [33.9975°N; 86.7494°W]	ALZ	-	-	1/1	301161
	Lake Waccamaw, NC, USA [34.3066°N; 78.4957°W] ***Ancylus* (*F.*) *hendersoni* (syn. *F. fragilis*) type locality**	NCW	-	-	2/2	301175
	North Saluda River at Les Mullinax County Park, northeast of Lima, Greenville Co., South Carolina [35.1271°N; 82.4264°W]	SCA	2/1	-	2/1	301187
	Potato Creek, southwest of Davis Station, Clarendon Co., South Carolina [33.5735°N; 80.2926°W]	SCP	2/1	-	2/1	301183
	Salkehatchie River, Barnwell Co., South Carolina [33.2370°N; 81.4020°W]	SCS	-	1/1	-	300228
	Laurel Lake, just west of Mattituck, Suffolk Co., New York [40.9778°N; 72.5562°W]	NYL	1/1	2/1	-	301147
	Lake Morey, Fairlee, Orange Co., Vermont [43.9264°N; 72.1417°W]	VTL	1/1	1/1	1/1	301148
	Mill Pond, Windsor, Windsor Co., Vermont [43.4761°N; 72.3976°W]	VTM	1/1	1/1	1/1	301149
	Sweets Pond, Mansfield, Bristol Co., Massachusetts [41.9895°N; 71.2550°W]	MAS	1/1	1/1	-	301200
	Quinebaug River, Plainfield, Windham Co., Connecticut [41.7471°N; 71.9142°W]	CTQ	-	1/1	1/1	301150
	Penobscot River, northwest of Medway, Penobscot Co., Maine [45.6149°N; 68.5392°W]	MEP	1/1	2/1	-	301151
	Little Falls Branch, Maryland [38.9441°N; 77.1169°W]	MDL	-	1/1	6/1	301186
	Impoundment of North Mosquito Creek at Cypress Cove Nature Park, Gadsden Co., Florida [30.7358°N; 84.8047°W]	FLM	-	1/1	1/1	301185
	São Miguel Island, Azores	AZOR	1/1	1/1	1/1	301196
	Basin of Arcachon, south of Le Teich, France	France	-	4/1	-	301197
	Subsidence pond in Upper Silesia, southern Poland	Poland	-	4/1	-	300279
	Chi-Chi, Nantou Co., Taiwan	Taiwan	-	2/1	2/1	300278
	Alimodian, Iloilo Prov., Panay Island, Philippines	Philippines	-	2/1	2/1	300276

(continues)

(continued)

Taxon	Locality [with coordinates if known]	Code	28S	COI	ITS2	Catalog #
F. sharpi	Lulumahu Stream, Nuuanu Valley, Oahu, Honolulu Co., HI [21.3461°N; 157.8209°W]	HIO	-	1/1	1/1	301191
Laevapex fuscus	Osage Creek, Benton Co., Arkansas [36.1972°N; 94.3377°W]	ARO	1/1	-	-	301195
	Marsh at the south end of Lake Maxinkuckee, Culver, Indiana [41.1839°N; 86.3850°W]	INM	2/1	-	-	301177
	Perry Lake at Perry Lakes Park, Marion, Perry Co., Alabama [32.6974°N; 87.2434°W]	ALP	1/1	-	-	301163
	Corn Creek Shoals, Coosa River, Elmore Co., Alabama [32.5519°N; 86.2011°W]	ALS	1/1	-	-	301198
	Pickerel Lake, Washtenaw Co., Michigan [42.4097°N; 83.9838°W]	MIP	1/1	6/1	1/1	300206
	Passaic River near Two Bridges, New Jersey [40.8858°N; 74.2689°W]	NJP	1/1	-	-	301152
	Wading River, Mansfield, Massachusetts [41.9894°N; 71.2548°W]	MAW	1/1	-	-	301153
	Big Cypress Bend, Florida State Park, Collier Co., Florida [25.9418°N; 81.4695°W]	FLB	1/1	-	-	300218
	Lake Helen (Helena), Volusia Co., Florida [28.9850°N; 81.2303°W]	FLH	3/2	-	-	301189
	Impoundment of North Mosquito Creek at Cypress Cove Nature Park, Gadsden Co., Florida [30.7358°N; 84.8047°W]	FLM	6/1	-	-	301185
Hebetancylus excentricus	Eslava Creek, Mobile Co., Alabama [30.6793°N; 88.1464°W]	ALE	-	1/1	-	301165
	Eslava Lake, Mobile Co., Alabama [30.6787°N; 88.1529°W]	ALF	-	1/1	-	301167
	Lake Helen (Helena), Volusia Co., Florida [28.9850°N; 81.2303°W]	FLH	2/1	1/1	-	301188
	Lake Talquin, west of Tallahassee, Leon Co., Florida [30.4389°N; 84.5304°W]	FLT	6/1	-	-	301190
Uncancylus sp. (?)	Lago Ranco, Chile [40.1910°S; 72.2606°W]	CLL	1/1	-	-	UGSB 1201

TBR was used for the other datasets. For maximum likelihood (ML) analyses, the best-fit model for each of the four datasets (TVM+I+G for 28S and COI, TVM+I for ITS-2) was obtained in Modeltest 3.06 (Posada & Crandall, 1998). In each case, one of the MP trees was used as a starting tree with the TBR branch-swapping algorithm in PAUP*. Bootstrap support values were generated using a fast-heuristic search with 100 replicates. Bayesian searches were run for $1x10^6$ generations in MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003) using the same best-fit model as in the ML analyses. Posterior probabilities were calculated by creating a majority-rule consensus for every 100th tree after burn-in of the first 3000 trees using PAUP*. Parsimony networks for three mt COI tip-clades of North American *Ferrissia* were constructed using the statistical parsimony (Templeton et al., 1992) method in TCS 1.21 (Clement et al., 2000).

RESULTS

Molecular Phylogenies

The addition of a large number of novel North American *Ferrissia* 28S nuclear rDNA genotypes to the Walther et al. (2006a) dataset did not result in any significant topological changes (Fig. 3). The same two *Ferrissia* 28S lineages were recovered: one containing a single genotype common to *F. rivularis* and *F. parallela* specimens sampled across the continent; the other comprised of three genotypes, all recovered from *F. fragilis* specimens, the most common of which was also present in near-topotypic populations of putative *F. mc-*

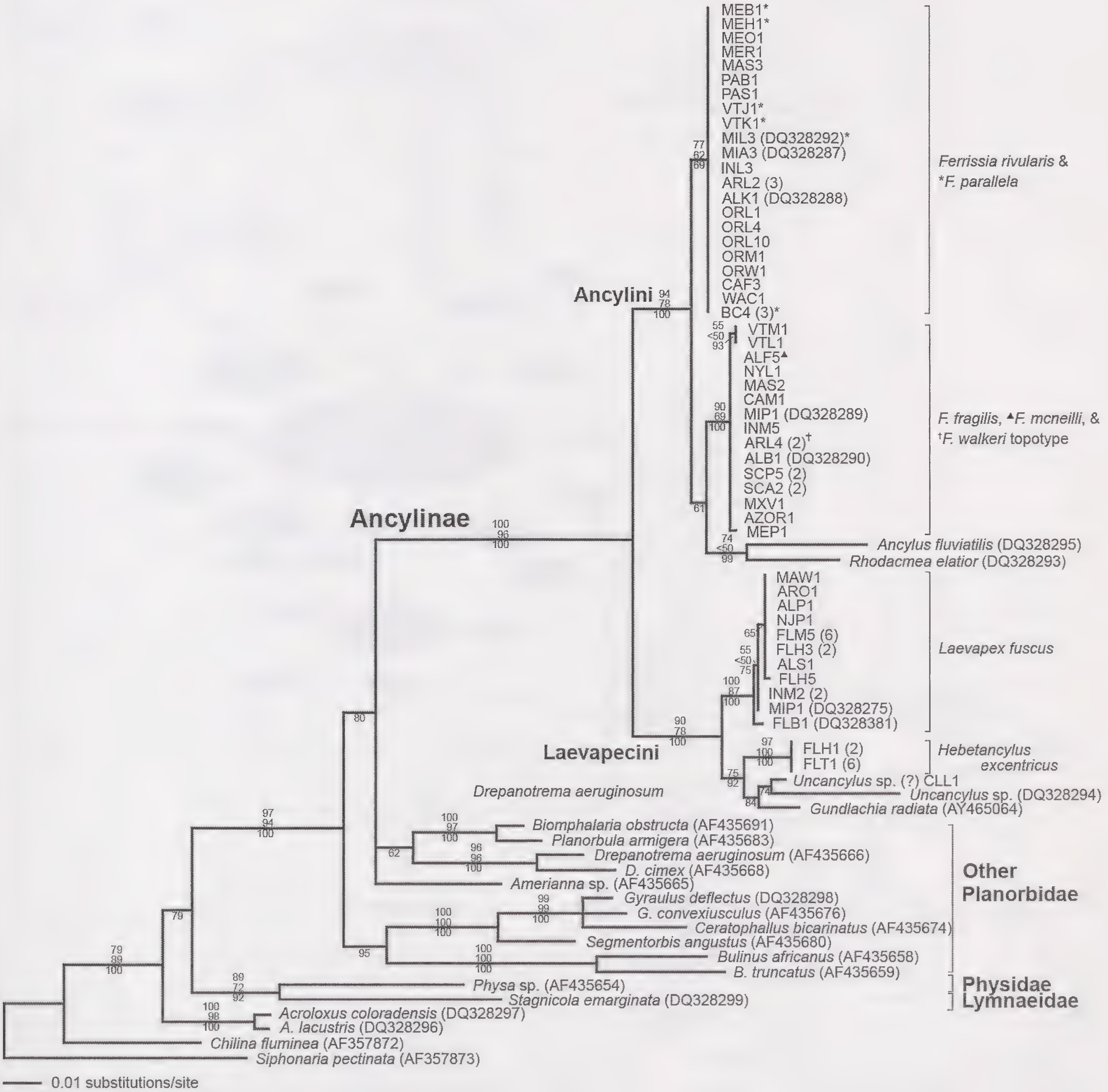


FIG. 3. Bayesian consensus phylogram for our nuclear 28S rDNA dataset. *Siphonaria pectinata* served as the outgroup, and non-novel genotypes obtained from GenBank are indicated by their terminal accession numbers. The taxonomic names and location codes are given for novel ancylinid sequences; Table 2 has detailed sampling location information. If >1 individual/location bore a genotype, this number is indicated in parentheses. In the *F. fragilis* clade, SCA2 (2) represents the two genotyped limpets sourced from Dillon & Herman's (2009) North Saluda River study population. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented.

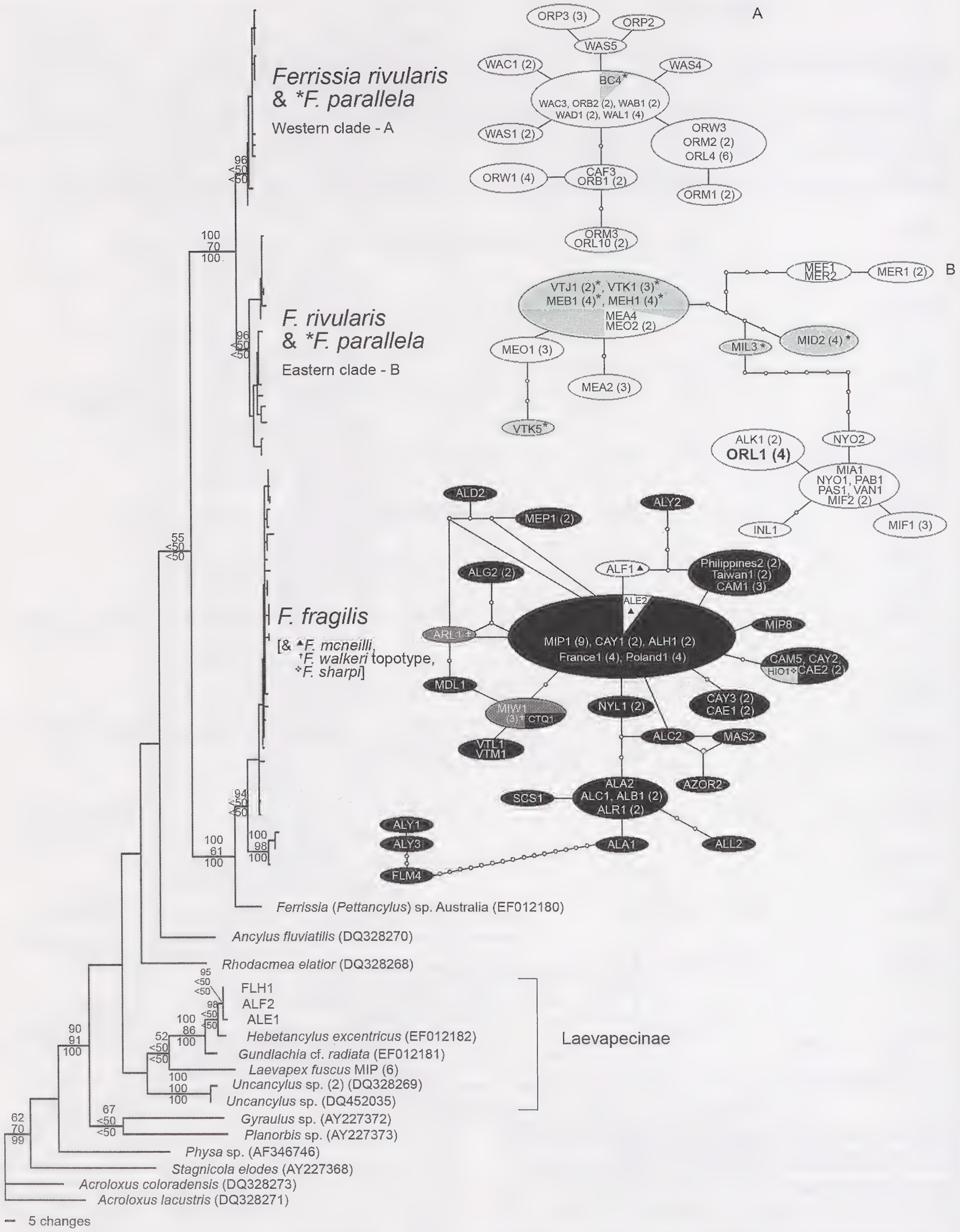


FIG. 4. A maximum parsimony phylogenetic tree for the North American *Ferrissia* species mt COI dataset presented together with unrooted statistical parsimony networks for each of the mt COI tip clades. The tree topology shown was one of the 5000 most parsimonious trees saved, but it only differed in within-tip clade branching order from the strict consensus tree. *Acroloxus lacustris* and *A. coloradensis* served as outgroups. Table 2 has location codes for all novel genotypes. If >1 individual/location bore a genotype, this number is indicated in parentheses. Non-novel genotypes obtained from GenBank are indicated by their terminal accession numbers. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented. In the unrooted networks, the size of each oval corresponds to the total number of individuals sharing a haplotype.

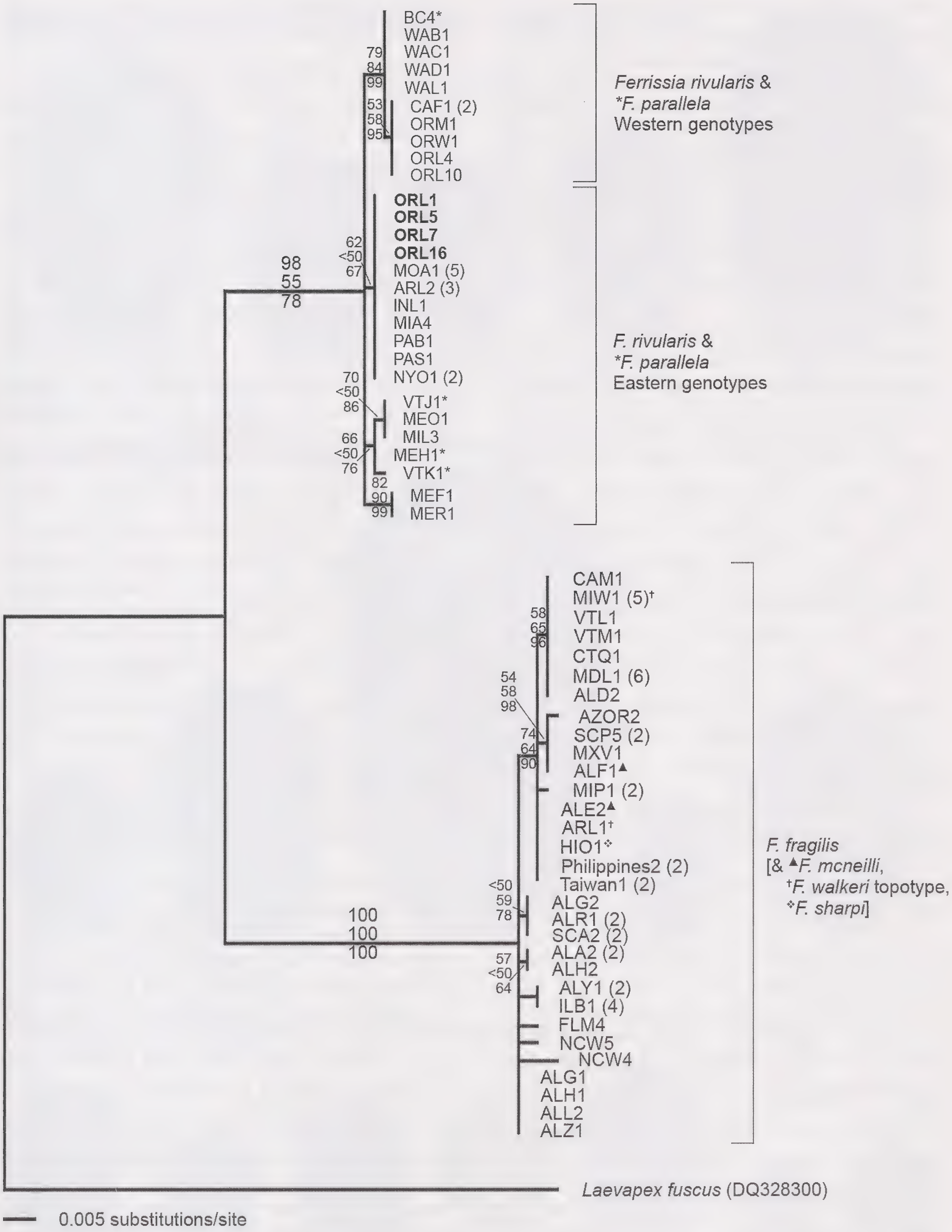


FIG. 5. Bayesian phylogram of the nuclear second internal transcribed spacer (ITS-2) dataset with *Laevapex fuscus* designated as the outgroup. Table 2 has sampling location codes information. If > 1 individual/location bore a genotype, this number is indicated in parentheses. In the *F. fragilis* clade, SCA2 (2) represents the two genotyped limpets sourced from Dillon & Herman's (2009) North Saluda River study population. Bayesian posterior probabilities appear below the branches with maximum parsimony (top) and maximum likelihood (middle) bootstrap support values also presented. Four Oregonian specimens that share a *Ferrissia rivularis* Eastern genotype are in bold.

neilli and *F. walkeri*. As in the previous study, the two *Ferrissia* lineages were paraphyletic for this marker, the *F. fragilis* clade being weakly sister with the tip *Ancylus/Rhodacmea* tip clade for maximum parsimony, maximum likelihood and Bayesian analyses. The only other significant addition to the Walther et al. (2006a) 28S dataset was the inclusion of the *Hebetancylus excentricus* genotypes (Fig. 3). This placed within the Laevapecini clade, but sister to South American *Uncancylus* and *Gundlachia* taxa rather than the co-occurring North American *Laevapex fuscus*.

The large nuclear ribosomal (28S) gene is relatively conserved, and much finer-scale phylogenetic resolution can be provided by protein-coding mitochondrial genes (Lee & Ó Foighil, 2005) and, to a lesser degree, by faster-evolving internal transcribed spacer (ITS) regions of the nuclear ribosomal gene complex (Hillis & Dixon, 1991; Lee & Ó Foighil, 2005). We genotyped our limpet samples for mt COI and ITS-2 markers and found that their phylogenetic analyses (Figs. 4, 5) did not change the primary conclusions evident in our 28S ribosomal trees (Fig. 3). Two highly distinctive clades of North American *Ferrissia* were also recovered for each marker; one composed of limpets identified as *F. rivularis* and *F. parallela*, the other containing *F. fragilis* and near-topotypic populations of putative *F. mcneilli* and *F. walkeri*.

The higher resolution provided by mt COI did yield qualitatively new phylogenetic information in the form of conspicuous geographic structuring within the *F. rivularis/parallela* clade (Fig. 4). Both nominal taxa in Pacific drainages formed an exclusive mt COI Western Clade, whereas specimens in eastern drainages formed a corresponding Eastern Clade (Figs. 4, 5). Note that the mt genetic structuring was geographic rather than taxonomic: within each regional clade, individual haplotypes were shared by limpets identified, according to Basch's (1963a) criteria, as *F. rivularis* and *F. parallela* (Figs. 4, 5). The only exception to this regional structuring was one western sampling site (ORL; Table 2) where some *F. rivularis* specimens carrying Eastern Clade haplotypes were recovered in sympatry with Western Clade conspecifics. Although sympatric ORL limpets bearing the respective mt lineages appeared morphologically indistinguishable (Fig. 6), nuclear ITS-2 genotypes corroborate the mt results. *Ferrissia rivularis* and *F. parallela* specimens bearing Western Clade mt genotypes produced an exclusive ITS-2 tip clade (Fig. 5), and there was agreement among mt and nuclear markers for six ORL *F. rivularis* (2 Western Clade, 4 Eastern Clade) genotyped for both COI and ITS-2.

The mt COI dataset incorporated sequences from a wide variety of ancylinid taxa and one of these, a *Ferrissia* (*Pettancylus*) sp. haplotype sampled from Queensland, Australia (EF012180; Albrecht et al., 2007), was robustly sister to the predominantly *Ferrissia fragilis* clade (Fig. 4). However, basal Ancylini mt COI nodes were poorly supported irrespective of the phylogenetic optimality criterion employed. In maximum parsimony trees, the predominantly *Ferrissia fragilis* clade + *Ferrissia* (*Pettancylus*) sp., was weakly (BS = 55) sister to *F. rivularis* (Fig. 4), although it was weakly (PP = 57) sister to an *Ancylus/Rhodacmea* tip clade in Bayesian and maximum likelihood trees.

Limpets matching *F. fragilis*'s general conchological description (Tryon, 1863; Basch, 1963a) occurring throughout North America in lotic (creeks and rivers) as well as lentic (ponds and lakes) habitats, formed a robustly supported shallow clade (Figs. 3–5). It also included genotyped specimens from near-topotypic populations of putative *F. walkeri* (Benton Co., Arkansas; Berrien Co., Michigan) and putative *F. mcneilli* (Eslava River and Eslava Lake, Alabama), both of which shared individual mt COI haplotypes with *F. fragilis* specimens (Fig. 4). Comparison of our genotyped Eslava specimens with those collected there by McNeill and identified by Walker as *F. mcneilli* showed that they possessed overlapping shell phenotypes (Fig. 7).

The predominantly *F. fragilis* clade lacked obvious geographic structuring, and Alabama samples were present throughout the mt COI and nuclear ITS-2 topologies (Figs. 4, 5). A notable feature, however, was the presence of multiple founder populations in Europe and Asia (Figs. 4, 5), discussed in detail by Walther et al. (2006b). Our novel data detected two additional founder populations on oceanic island archipelagoes: Azorean *Ferrissia* and our single specimen of *F. sharpi*. The latter taxon, a Hawaiian putative endemic, shared a mt COI haplotype with multiple Californian *F. fragilis* specimens, though it was distinct from the shared haplotype linking another subset of Californian *F. fragilis* with founder populations in Taiwan and the Philippines (Fig. 4).

Clade-Specific Shell Phenotypes

Although all three genes employed readily differentiated the two main lineages of *Fer-*

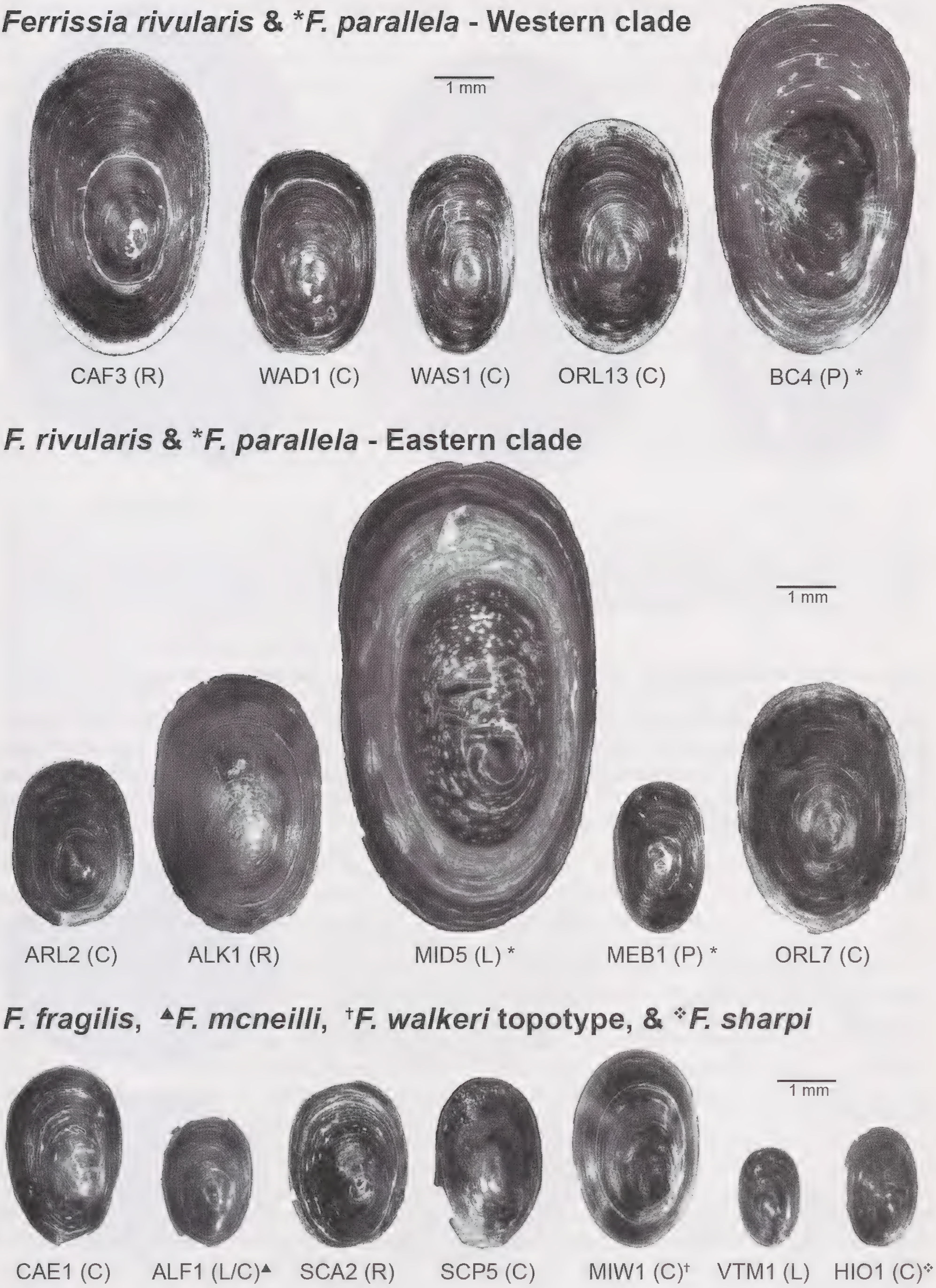
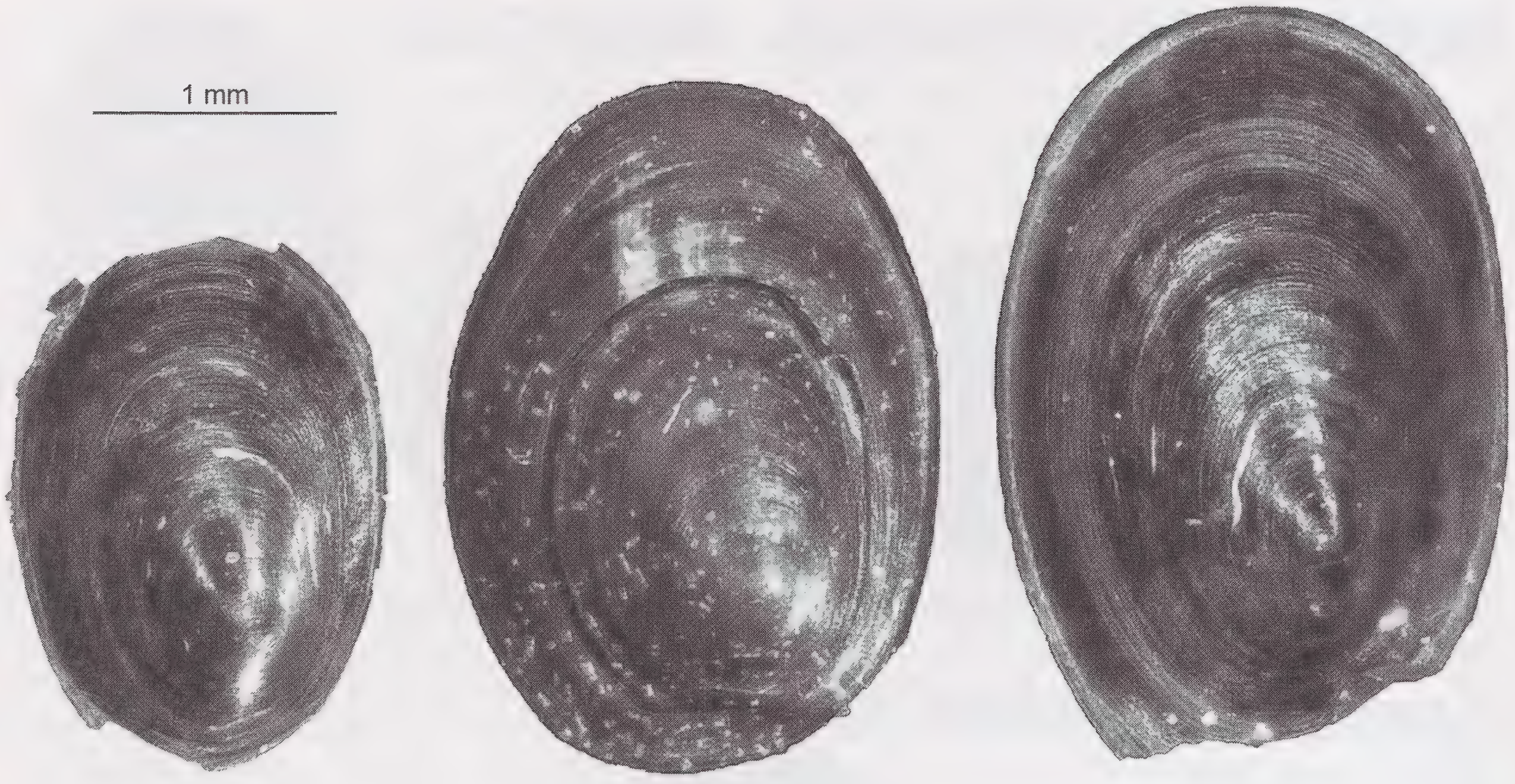


FIG. 6. Photographs of representative shell vouchers arranged according to their topological placement in mt COI and ITS-2 gene trees (Figs. 4, 5). Table 2 has corresponding location codes. Letters in parentheses accompanying the codes indicate habitat sampled: R = river, C = creek or stream, L = lake, P = pond, L/C = creek mouth emptying into a lake.



F. fragilis ALF1 *F. mcneilli* UMMZ 161631 *F. fragilis* ALF5

FIG. 7. Comparative photographs of a representative *Ferrissia mcneilli* specimen sampled by L. H. McNeill at Eslava Creek, Alabama, and identified by Walker (UMMZ 161631), juxtaposed with two genotyped Eslava Creek, Alabama, limpets sampled by the authors and identified by them as *F. fragilis*.

rissia in North America (Figs. 3–5), we were interested in identifying morphological attributes that could more conveniently serve this purpose. Differentiating adult members of the *F. rivularis/F. parallella* clade was easy because their maximum body size (≤ 9 mm in length; Fig. 6) was much larger than the predominantly *F. fragilis* clade members we genotyped (≤ 3.5 mm in length; Fig. 6), consistent with Tryon's (1863) description of *F. fragilis* as being "much the smallest of our species". This still leaves the difficulty of identifying the cladal affinities

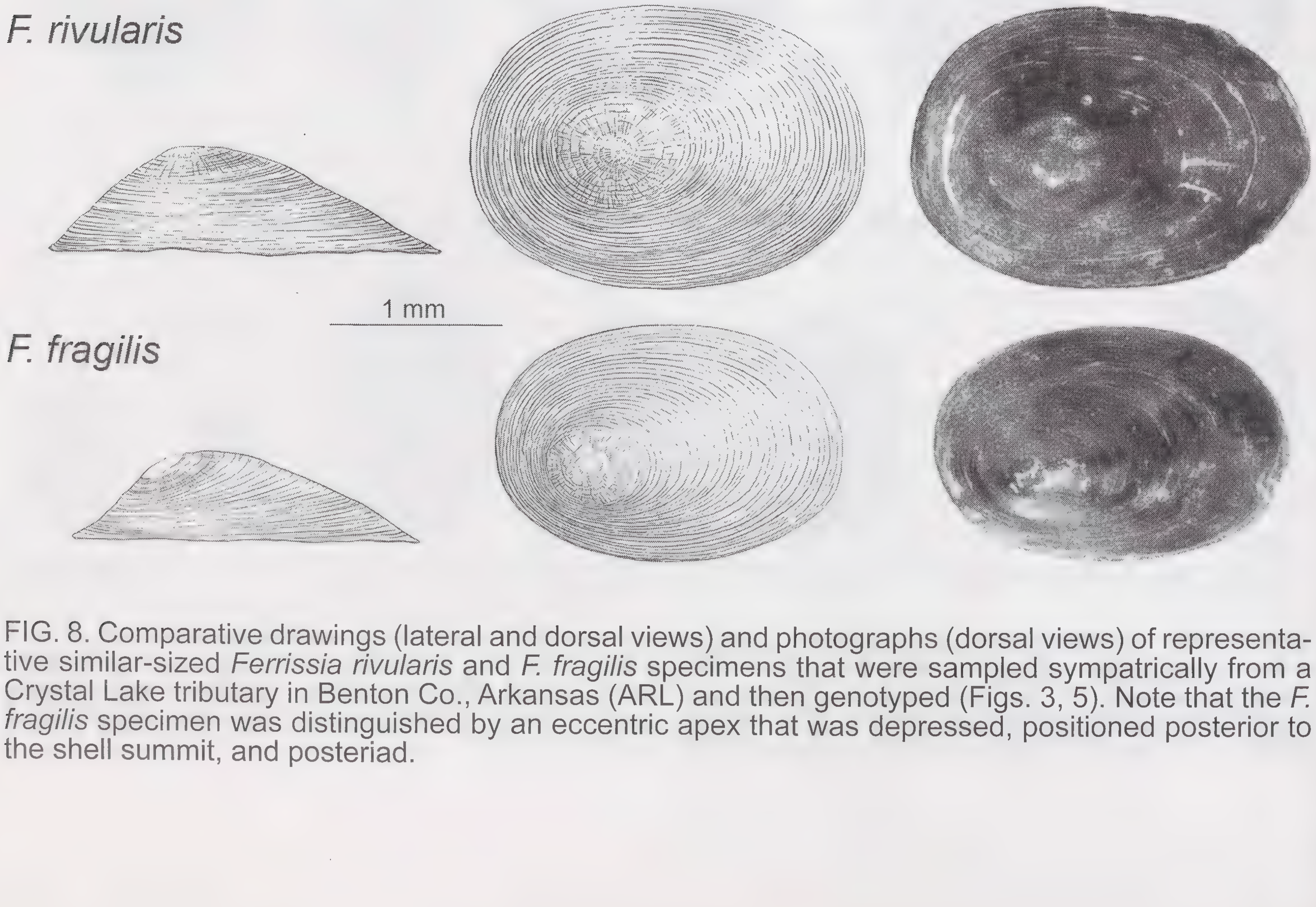


FIG. 8. Comparative drawings (lateral and dorsal views) and photographs (dorsal views) of representative similar-sized *Ferrissia rivularis* and *F. fragilis* specimens that were sampled sympatrically from a Crystal Lake tributary in Benton Co., Arkansas (ARL) and then genotyped (Figs. 3, 5). Note that the *F. fragilis* specimen was distinguished by an eccentric apex that was depressed, positioned posterior to the shell summit, and posteriad.

TABLE 3. Apical descriptions of the nominal taxa present in our predominantly *Ferrissia fragilis* clade (Figs. 3–5).

Taxa	Salient Apex Characteristics	Author
F. fragilis	"elevated, acute, curved backwards, with about 2/3rds of the shell anterior to it"	Tryon, 1863
F. walkeri	"excentric...decidedly more so than in *A. rivularis*...summit of the shell is in front of the somewhat depressed apex"	Pilsbry & Ferriss, 1907
F. michiganensis (syn. *F. walkeri* by Basch, 1963a)	"the greatest height being about in the center of the shell, from which point it slopes slightly towards the apex....apex slightly depressed, excentric, turned toward the right side"	Winslow, 1923
F. mcneilli	"prominent, sub-acute, somewhat obtuse, eccentric..." (lateral-view figure shows that summit of shell is in front of depressed apex)	Walker, 1925
F. sharpi	"well behind the middle and well to the right of the median line...inclined to the right and posteriad" (lateral-view figure shows that summit of shell is in front of depressed apex)	Hubendick, 1967

of smaller-bodied (≤ 4 mm in length) North American *Ferrissia* specimens.

Detailed descriptions of the nominal taxa present in our predominantly *Ferrissia fragilis* clade (Figs. 3–5) agree on a number of potentially useful distinguishing features of the shell apex (Table 3). These include a more pronounced apex eccentricity (relative to *F. rivularis*) in addition to a depressed/posteriad orientation associated with its positioning posterior to the shell summit. These features, especially the latter, are apparent in the type material of nominal *F. fragilis*, *F. walkeri*, and *F. mcneilli* (Fig. 2), as well as in our voucher material of genotyped *F. fragilis*, *F. sharpi,* and near-topotypic putative *F. walkeri* and *F. mcneilli* (Fig. 6). Although this appeared promising, the most meaningful test necessarily entailed sympatric populations: could we predict *Ferrissia* cladal membership based on apical differences of co-occurring similarly-sized limpets? Two of our sampling sites (ARL and MAS) contained limpets from both clades (Fig. 1; Table 2) and two distinctions were evident among their respective shell phenotypes. First, *F. fragilis* clade member shells were (as the specific name implies) more fragile than that of sympatric *F. rivularis* clade members, and many of the former were inadvertently damaged by routine handling during sampling and/or tissue extraction. Second, although there was some minor individual variation, adult *F. fragilis* clade members had apices (protoconchs) that were typically positioned just posterior to the shell summit (at the shell summit for *F. rivularis* clade members) and that were consistently more eccentric, depressed and posteriad in orientation than those of similarly-sized sympatric *F. rivularis* clade members (Fig. 8).

DISCUSSION

Our primary phylogenetic result was consistent for all three genetic markers: two highly distinctive *Ferrissia* clades, each encompassing multiple nominal species, were widely distributed across North American watersheds. In a major departure from Basch's (1963a) taxonomic scheme, both clades lacked discrete habitat demarcations and occurred in both lentic and lotic habitats.

Nominal *Ferrissia rivularis* and *F. parallela* specimens shared individual genotypes for all three markers and exhibited geographic, rather than taxonomic, genetic structuring. Our data therefore strongly indicated that the two nominal taxa represent lotic (*F. rivularis*) and lentic (*F. parallela*) ecophenotypes of the same limpet species. *Ferrissia rivularis* (Say, 1817) has priority over *F. parallela* (Haldemann, 1841), and we recommend that the latter taxon be reclassified as a *F. rivularis* junior synonym. This result was not entirely surprising. because Basch (1983a) regarded *F. parallela* as "a direct descendant of *F. rivularis*". However, we cannot be confident that lentic environments represent derived habitats for *F. rivularis*, because, at present, we lack convincing sister lineages for this North American species that would allow us to infer a plesiomorphic habitat type.

The recovery of east-west genetic structuring in our sampled *F. rivularis* populations for mt COI and, to a lesser extent, for nuclear ITS-2, was consistent with the presence of a long-term barrier to gene flow, presumably along the continental divide. Nevertheless, our discovery of limpets bearing Eastern genotypes in one of the Western populations indicated that this barrier has been secondarily breached in an east-to-west direction, possibly *via* human agency. Preliminary genetic characterization of the genetically mixed east/west population (ORL) did not recover evidence of introgression, for example, cyto/nuclear disjunction. However, this does not necessarily imply that the two morphologically indistinguishable lineages are reproductively incompatible because our sample sizes were small and some ancylinids reproduce predominantly by self-fertilization (Städler et al., 1993, 1995; Dillon & Herman, 2009). The geographically widespread European ancylinid *Ancylus fluviatilis* encompasses a cryptic species complex containing multiple reproductively isolated lineages (Pfenniger et al., 2003; Albrecht et al., 2006). Future in-depth research of continental *F. rivularis* populations may well support a similar conclusion, but at the present state of our knowledge, we consider it most useful to classify the observed east-west genetic structuring as within-species phylogeographic variation.

Nominal *Ferrissia fragilis*, *F. sharpi* and putative *F. mcneilli* and *F. walkeri* specimens shared individual genotypes (Figs. 3–5) and exhibited overlapping shell phenotypes (Figs. 6, 7; Table 3). These results led us to conclude that they are all conspecific and to reclassify *F. sharpi* (Sykes, 1900) and *F. walkeri* (Pilsbry & Ferriss, 1907) and *F. mcneilli* Walker, 1925, as junior synonyms of *F. fragilis* (Tryon, 1863).

Ferrissia fragilis has a most interesting, multilayered biogeography. Our mt COI gene trees recovered a robust sister relationship with an Australian *Ferrissia* (*Pettancylus*) sp. (Fig. 4), thereby corroborating the preliminary Old World phylogenetic linkage of *F. fragilis* with an African sample of *Ferrissia* (*Pettancylus*) cf. *clessiniana* obtained for a different mt marker (Walther et al., 2006b, c). Within North America, *F. fragilis*, unlike *F. rivularis*, showed no strong evidence of regional cladogenic structuring, for example, the most common mt COI haplotype was present in Alabama, Michigan, and California samples (Fig. 4). However, the observed genetic diversity was unevenly distributed: southeastern (Alabama) samples were the most diverse and encompassed genotypes that placed throughout the topologies of ITS-2 and mt COI gene tree/network topologies (Figs. 4, 5). Another notable feature was the presence of a discrete subsample of North American mt COI haplotypes, or near-mutational derivates, in European, Asian and oceanic island founder

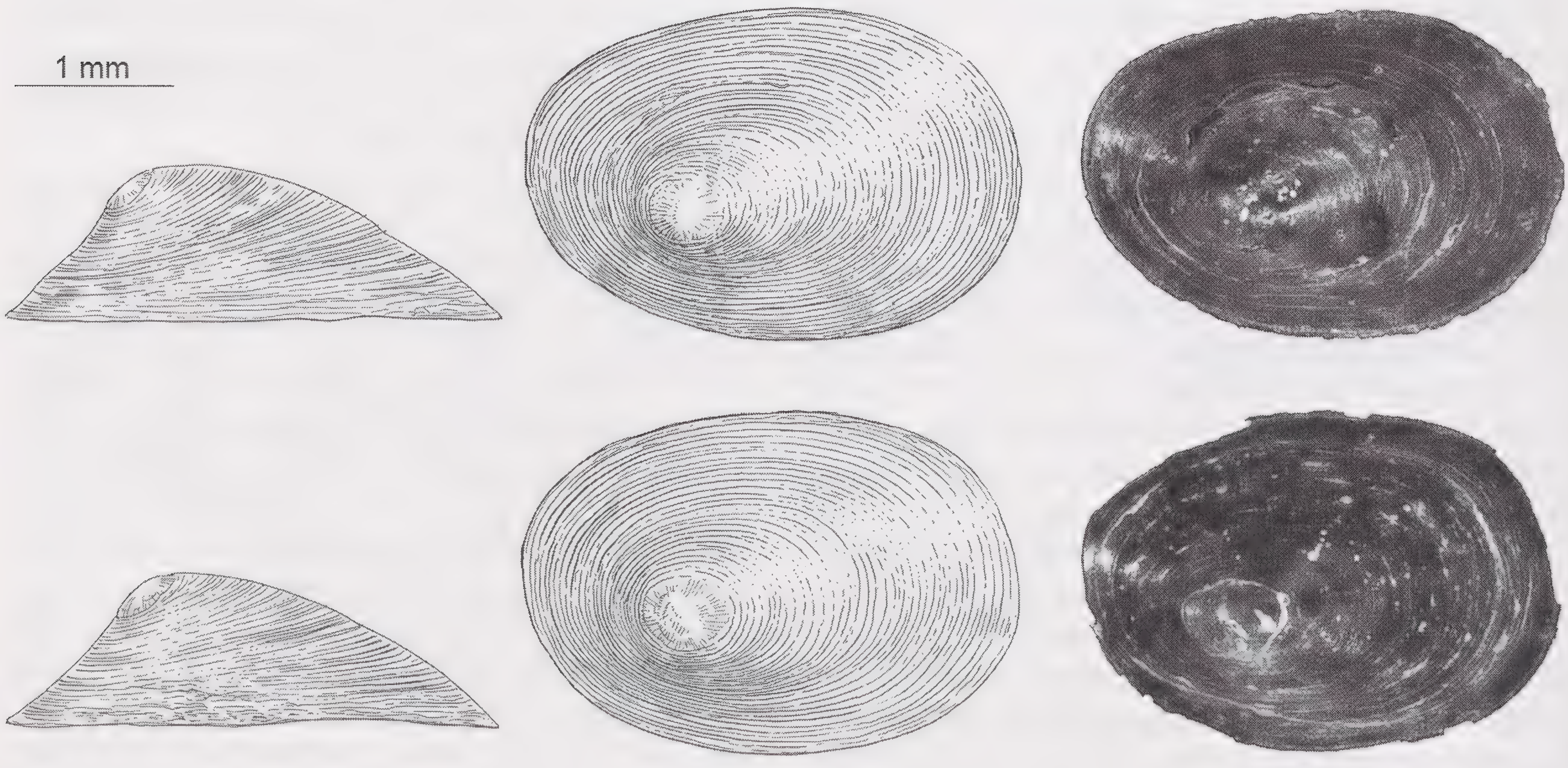


FIG. 9. Drawings (lateral and dorsal views) and photographs (dorsal views) of the shells of two genotyped (SCA2; Figs. 3, 5) North Saluda River *F. fragilis* specimens. Note that they display eccentric, depressed, posteriad apices that are positioned posterior to the shell summit.

populations (Fig. 4) that apparently stem from historically recent cryptic anthropogenic invasions (Walther et al., 2006b, c). In some cases, our expanded sampling of North American populations allowed us to identify putative regional source populations, for example, Hawaiian and Asian (Taiwan, Philippines) founder populations exclusively shared (different) haplotypes with Californian conspecifics (Fig. 4).

Putting aside recent globally invasive founder populations, the deeper phylogenetic Old World ties of *Ferrissia fragilis* to Australian and African congeners (Fig. 4; Walther et al., 2006b, c) ostensibly contradict Hubendick's (1964) distinction of New World *F.* (*Ferrissia*) from Old World *F.* (*Pettancylus*) subgenera. However, Hubendick's (1964) anatomical analysis of New World *Ferrissia* taxa was restricted to *F. rivularis* and *F. parallela*, and he apparently did not examine North American populations of *F. fragilis*. Nevertheless, he did study what we now know to be cryptic invasive *F. fragilis* founder populations in Hawaii (Hubendick, 1967; identified as *F. sharpi*) and in Europe (Hubendick, 1964; identified as *Watsonula wautieri*), both of which he assigned to *Pettancylus*. This agreement among molecular phylogenetic (Fig. 4; Walther et al., 2006b, c) and morphological diagnoses (Hubendick, 1964, 1967) concerning *F. fragilis*' systematic affinities allow us to group it with the Old World subgenus *Pettancylus*. However, this necessitates a subgeneric name change because Hannibal (1912) used *F. fragilis* as the type species for his new subgenus *Kincaidilla*, and this has precedence over *Pettancylus* Iredale 1943. The *Ferrissia* subgenus *Kincaidilla* notably unites, for the first time, New World and Old World septum-forming *Ferrissia* taxa.

Our systematic conclusions are obviously incongruent with Dillon & Herman's (2009) recent synonymization of *F. rivularis* and *F. fragilis* based on allozymic and morphometric analyses of two South Carolina populations. They had kindly forwarded exemplar specimens to us for genotyping, and it is important to stress that their allozyme analyses concurred with our DNA phylogenies: both populations were found to be conspecific. The disagreement centers on the taxonomic identity of one of their two study populations: North Saluda River.

Dillon & Herman (2009) identified their North Saluda River study population as *F. rivularis* partially on ecophenotypic conchological features but also on the basis of habitat (a fast flowing stream segment). However, *F. fragilis* population-level conchological phenotypes span a wide vertical spectrum from depressed to elevated (Basch 1963a: fig. 18), and we now know that this species occurs in many lotic habitats across the continent (Table 2; Fig. 6). Dillon & Herman (2009) justified their synonymization of *F. rivularis* and *F. fragilis* on the presumed absence of any reliable morphological distinction separating these two taxa. Nevertheless, a suite of morphological distinctions including maximum body size (Basch, 1963a; Fig. 6), details of apical positioning and orientation (Table 3; Fig. 8), egg capsule characteristics (Basch, 1959), and the inducible ability to form a septum (Basch, 1963a; Richardot, 1974, 1977a, b), give grounds for cautious optimism that it should be possible to distinguish sympatric populations without resorting to genotyping.

The available morphological data (body size, egg capsule characteristics, conchology) for North Saluda River specimens were consistent with their phylogenetic identity (Figs. 3, 5). For instance, their maximum length of 3.4 mm, (Dillon & Herman, 2009), fell comprehensively short of that expected for a *Ferrissia rivularis* adult year class (Basch,1963a: fig. 13). Their egg capsules exclusively contained single eggs (Dillon & Herman, 2009), a characteristic of *F. fragilis* (Basch, 1959; Mirolli, 1960; Walther et al., 2006b) but not of *F. rivularis* and *F. parallela* (Basch, 1959; Burky, 1971; Clarke, 1973). Finally, they clearly displayed the apical characteristics of *F. fragilis* (Fig. 9). In conclusion, the available phylogenetic, morphological and reproductive characters concur that Dillon & Herman's (2009) North Saluda River study population was *F. fragilis*, not *F. rivularis*, and we are therefore unable to support the proposed synonymization of these two taxa.

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FRESHWATER GASTROPOD PROVINCES FROM ARGENTINA

Verónica Núñez*, Diego E. Gutiérrez Gregoric & Alejandra Rumi

CONICET, División Zoología Invertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900, La Plata, Argentina

ABSTRACT

Diverse biogeographic regions have been proposed in Argentina using different approaches of biogeography and different criteria. However, none of these proposals has considered freshwater gastropods in estimating biodiversity values, although gastropods from continental waters have great ecological importance and are a useful group to characterize freshwater environments. For this reason, our aim is to define freshwater gastropod provinces and to compare them with the available information on biogeographic regions previously defined using other data sets. The gastropod database comprises approximately 4,000 georeferenced records from museum collections, bibliographic records, and collections of our research team. A similarity analysis among basins was carried out using the species as characters and applying Jaccard's coefficient and UPGMA clustering with the MVSP program to obtain the resulting phenogram. Eight provinces were identified: I, Misionerean; II, Middle Paraná; III, Uruguay River; IV, Lower Paraná – Río de la Plata; V, Central; VI, Cuyo; VII, Northern Patagonia; and VIII, Southern Patagonia. Between Provinces V and VI lies a Transitional Zone that shows a higher faunal similarity with the Del Plata basin than with the Patagonian basins. The provinces with the highest diversity were I and IV (H = 1.92 and 1.89 respectively), while Province III shows the highest richness (S = 51) and quantity of endemic and vulnerable species. These provinces are the most densely populated in the country and therefore include the areas with the highest environmental degradation level.

Key words: Argentina, freshwater Gastropoda, diversity, endemism, provinces, richness.

INTRODUCTION

Gastropod faunas from continental waters are ecologically important in many aquatic ecosystems; more specifically, they have an essential trophic function in the dynamics of epicontinental aquatic environments. Because of their slow movements, adequate size, and large population numbers, they are an appropriate group to characterize freshwater environments. They constitute a group very rich in species with a tendency to local endemism. Moreover, freshwater gastropods are promising tools as pollution indicators by means of assessments of mollusk community composition and biological monitoring programs that rate water quality and status of aquatic biotopes based on invertebrate assemblages (Strong et al., 2008).

Recently, Strong et al. (2008) analyzed the world's gastropod fauna diversity and proposed a spatial distribution of species. Biogeography of Neotropical aquatic continental gastropods is poorly known. Unlike many terrestrial species, which can disperse widely in suitable habitats, the spatial range of freshwater species tends to correspond to present or formerly continuous river basins or lakes. In many instances, the range within a system will also be restricted by particular habitat requirements (*World Conservation Monitoring Centre* – WCMC, 1998).

Freshwater gastropods have been preliminarily used to define local or regional diversity in Argentina (Rumi et al., 2006, 2008). Since then, our knowledge about systematics and diversity of the Argentinean freshwater gastropod fauna has increased substantially, allowing us to adjust and improve those regional proposals.

Argentina covers an area of 2,780,403 km^2 of southern South America, and is about 3,900 km long from north to south. In addition, its variation in altitude favors the development of a great variety of environments, from subtropical rainforests to Patagonian steppes, being one of the countries in the world with the highest biogeographic diversity (Bertonatti & Corcuera,

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*Corresponding author: nmariaveronik@yahoo.com.ar

2000). The Del Plata basin and the Patagonian System are the most important hydrographic systems of Argentina. According to estimates carried out by WCMC (1998), the Del Plata basin is identified as one of the 27 areas of special importance for freshwater mollusk diversity; they are key hotspots of diversity with high rates of endemism among freshwater gastropods.

Diverse divisions into biogeographic regions have been proposed for the Argentinean territory using different approaches of biogeography and other criteria. Such are the zoogeographic sketches of Morrone (2002, 2006) and the ecoregions described by Burkart et al. (1999) on the basis of climatic variables and particular ecologic functional features, mainly considering land flora and fauna. In all these schemes, the terms subregion, territory, unit or area are used indiscriminately. Freshwater gastropods are not taken into account in estimating biodiversity values. However, some of these proposals are related to the freshwater environments. According to Bonetto (1994), two biogeographic territories – with a Transitional Zone between them – are defined on hydrographic basins. Canevari et al. (1999) described six wetland regions. Based on fish distribution, Ringuelet (1975) proposed three ichthyologic provinces, whereas López et al. (2002) defined 11 ecoregions.

Our objective here is to define freshwater gastropod provinces and to compare them with the information available on biogeographic regions formerly defined for Argentina, mainly with those related to freshwater environments. This work is part of a long-range project, which also includes bivalves in similar regional analyses.

MATERIAL AND METHODS

Our database, with 101 species of gastropods, was assembled for research on the systematics, biogeography, and diversity of freshwater mollusks, and was published by Rumi et al. (2006, 2008). It comprises approximately 4,000 georeferenced records from: collections housed in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN), Museo de La Plata, La Plata (MLP), and Fundación "Miguel Lillo", Tucumán (FML), bibliographic citations, and collections of our research team.

Three species included in this database were not considered for this study: (a) *Laevapex* sp. (Ancylidae), because it is unidentified; (b) *Heleobia australis* (d'Orbigny, 1835), and (c) *H. conexa* (Gaillard, 1974) (Cochliopidae), both of which inhabit mainly seawater environments. On the other hand, two species were added to the database, a recently published species – *Chilina iguazuensis* Gutiérrez Gregoric & Rumi, 2008; Chilinidae – and another with a recent record for Argentina (*Galba truncatula* (Müller, 1774) – Lymnaeidae) (Bargues et al., 2006).

Because this work deals with freshwater organisms, we used hydrographic basins as operational geographical units. The basins used are those defined by the *Sistema Nacional de Información Hídrica de la Argentina* (2002), with some modifications; names of each basin and their description are given also by López et al. (2002). Two more basins were added due to their particular characteristics: basin no. 102 (streams in the Somuncurá System, Province of Río Negro) as in the work of López et al. (2002) and basin no. 103 (Martín García Island, Province of Buenos Aires) (Fig. 1). On the other hand, some basins were divided: no. 67 in 67a (small streams of SE Chubut Province) and 67b (small streams of E Santa Cruz Province), and no. 60 in 60a (upper Colorado River) and 60b (lower Colorado River). Only one of the three parts of basin no. 61 was considered (61a: small streams of SE Buenos Aires, to the N of Colorado River), because the rest of this basin has few records. Some basins were united to others, because they represent small adjacent basins of several independent drainage streams.

To recognize similarities between hydrographic basins, we performed a similarity analysis based on the native fauna of freshwater gastropods following the methodology used by López et al. (2002) for fish. A basic matrix of binary data (presence/absence) was laid out; basins were treated as Operational Taxonomic Units and species as characters. Basins with no records (38, 52, 58, 78, 81, 87 and 92) and basins with one or with two records (32, 60a, 61b–c, 71, 77, 80, 90, 95, 96 and 99; 70 and 101, respectively) were not included in the analysis. A total of 69 basins was used. Likewise, species found in only one basin were not included (Table 1). Thus, a total of 77 species of the 100 present in Argentina was considered. A resulting phenogram was obtained applying Jaccard's coefficient (Real

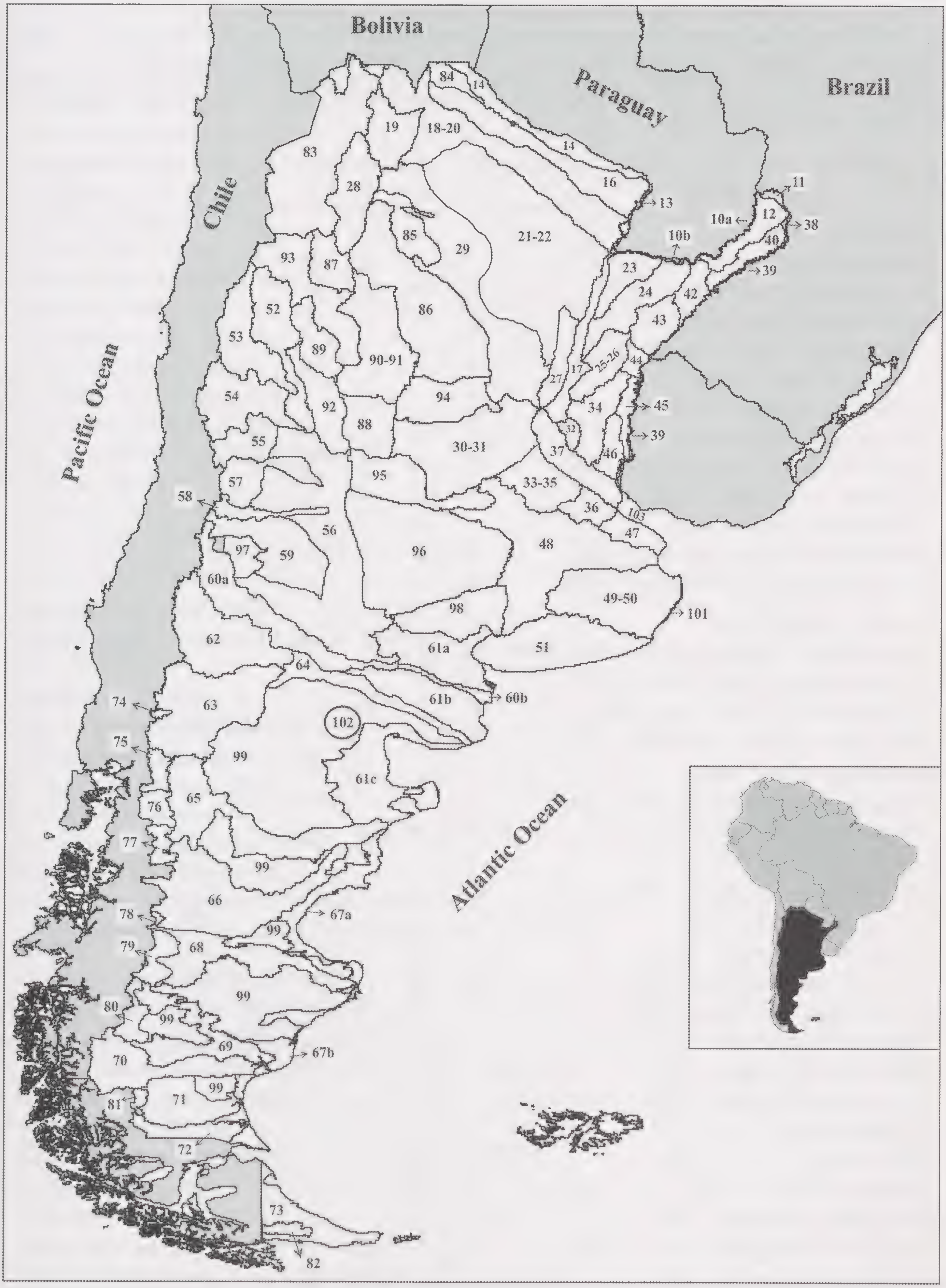

FIG. 1. Division of the Argentinean territory into its hydrographic basins.

TABLE 1. List of species and their distribution by provinces. *: Species that inhabits only one basin; +: Exotic species; both were not considered in similarity analysis; Provinces: I. Misionerean, II. Middle Paraná, III. Uruguay River, IV. Lower Paraná – Río de la Plata, V. Central, VI. Cuyo, VII. Northern Patagonia, VIII. Southern Patagonia; TZ: Transitional Zone; E: Endemic species of Argentina; V: Vulnerable species; EV: Endemic and vulnerable species; P: Non-endemic species present in each province. S: Species of sanitary importance.

Species/Provinces	I	II	III	IV	V	VI	VII	VIII	TZ
Ampullariidae Gray, 1824									
Asolene platae (Maton, 1809)			P	P					
A. pulchella (Anton, 1839)	P	P	P	P					
A. spixii (d'Orbigny, 1835)	P	P	P	P					
Felipponea elongata (Dall, 1921)			V						
F. iheringi (Pilsbry, 1933)			V						
F. neritiniformis (Dall, 1919)			P						
Marisa planogyra Pilsbry, 1933	P	P		P	P				
Pomacea canaliculata (Lamarck, 1822)	P	P	P	P	P	P			P
P. insularum (d'Orbigny, 1835)	P	P	P	P					
P. scalaris (d'Orbigny, 1835)		P	P	P					
Pomella americanista (Ihering, 1919)	P	P	P						
P. megastoma (G. B. Sowerby I, 1825)	P		P	P					
Thiaridae Troschell, 1857									
Aylacostoma chloroticum Hylton Scott, 1954*	EV								
A. guaraniticum (Hylton Scott, 1951)*	EV								
A. stigmaticum Hylton Scott, 1954*	EV								
Melanoides tuberculatus Müller, 1774+	P								
Cochliopidae Tryon, 1866									
Heleobia castellanosae (Gaillard, 1974)*				EV					
H. guaranitica (Doering, 1884)	P	P	P	P					
H. hatcheri (Pilsbry, 1911)						P	P	P	P
H. isabelleana (d'Orbigny, 1835)*				V					
H. kuesteri (Strobel, 1874)						EV			
H. montana (Doering, 1884)					EV				EV
H. occidentalis (Doering, 1884)		EV				EV			
H. parchappii (d'Orbigny, 1835)	P	P	P	P	P	P	P		P
H. peiranoi (Weyrauch, 1963)*					EV				
H. piscium (d'Orbigny, 1835)		P	P	P					
H. rionegrina (Gaillard, 1974)*									
H. sublineata (Pilsbry, 1911)*								EV	
H. tucumana (Gaillard, 1974)		EV			EV				EV
H. vianai (Parodiz, 1960)		EV				EV			
Lithoglyphidae Troschel, 1857									
Potamolithus agapetus Pilsbry, 1911			EV	EV					
P. buschii (Frauenfeld, 1865)			EV	EV					
P. callosus Pilsbry, 1925			P						
P. catharinae Pilsbry, 1911			V						
P. concordianus Parodiz, 1966			EV						

(continues)

(continued)

Species/Provinces	I	II	III	IV	V	VI	VII	VIII	TZ
P. conicus (Brot, 1867)			EV						
P. dinochilus Pilsbry, 1896			EV						
P. doeringi Pilsbry, 1911	V								
P. hidalgoi Pilsbry, 1896			EV						
P. iheringi Pilsbry, 1896			P						
P. lapidum (d'Orbigny, 1835)	P		P	P					
P. microthauma Pilsbry, 1896			V						
P. orbignyi Pilsbry, 1896			V	V					
P. paranensis Pilsbry, 1911	E	E	E						
P. peristomatus (d'Orbigny, 1835)	P	P	P						
P. petitianus d'Orbigny, 1840	P		P	P					
P. philipianus Pilsbry, 1911	P		P						
P. quadratus Pilsbry & Ihering, 1911			V						
P. rushii Pilsbry, 1896			E						
P. simplex Pilsbry, 1911			EV						
P. tricostatus (Brot, 1867)*			EV						
P. valchetensis Miquel, 1998*									
Glacidorbidae Ponder, 1986									
Gondwanorbis magallanicus (Meier-Brook & Smith, 1976)							V	V	
Chilinidae Gray, 1828									
Chilina aurantia Marshall, 1924*							V		
C. dombeiana (Bruguiere, 1789)							P		
C. fluminea (Maton, 1809)			P	P	P				
C. fulgurata Pilsbry, 1911							E	E	
C. gallardoi Castellanos & Gaillard, 1981			E	E					
C. gibbosa G. B. Sowerby I, 1841							P		
C. guaraniana Castellanos & Miquel, 1980*	EV								
C. iguazuensis Gutiérrez Gregoric & Rumi, 2008*	EV								
C. megastoma Hylton Scott, 1958*	EV								
C. mendozana Strobel, 1874						E			
C. neuquenensis Marshall, 1933							EV		
C. parchappii (d'Orbigny, 1835)					E	E			E
C. patagonica Sowerby, 1874							P	P	
C. perrieri Mabille, 1833							EV	EV	
C. portillensis Hidalgo, 1880		EV							EV
C. rushii Pilsbry, 1911			E	E					
C. strebeli Pilsbry, 1911							EV	EV	
Lymnaeidae Rafinesque, 1815									
Galba truncatula (Müller, 1774)*+						S			
Lymnaea columella Say, 1817+	S	S	S	S					
L. diaphana King, 1830							P	P	

(continues)

(continued)

Species/Provinces	I	II	III	IV	V	VI	VII	VIII	TZ
L. pictonica Rochebrune & Mabille, 1885								P	
L. plicata Hylton Scott, 1953*							EV		
L. viatrix (d'Orbigny, 1835)		S		S	S	S	S	S	S
Planorbidae Rafinesque, 1815									
Acrorbis petricola Odhner, 1937	V								
Antillorbis nordestensis (Lucena, 1954)	P	P	P	P	P				
Biomphalaria intermedia (Paraense & Deslandes, 1962)	P	P							
B. occidentalis Paraense, 1981		P							
B. oligoza Paraense, 1974		P			P				
B. orbignyi Paraense, 1975	P	P	P	P	P	P			P
B. peregrina (d'Orbigny, 1835)	S	S	S	S	S	S	S		S
B. straminea (Dunker, 1848)	S	S	S	S	S				
B. tenagophila (d'Orbigny, 1835)	S	S	S	S	S				S
Drepanotrema anatinum (d'Orbigny, 1835)	P	P	P	P	P				
D. cimex (Moricand, 1839)	P	P	P	P					
D. depressissimum (Moricand, 1839)	P	P	P	P					P
D. heloicum (d'Orbigny, 1835)	P	P		P	P				
D. kermatoides (d'Orbigny, 1835)	P	P	P	P	P		P		P
D. lucidum (Pfeiffer, 1839)	P	P	P	P					
Ancylidae Rafinesque, 1815									
Anisancylus obliquus (Broderip & Sowerby, 1832)					P		P		
Gundlachia ticaga (Marcus & Marcus, 1962)	V								
Hebetancylus moricandi (d'Orbigny, 1837)	P	P	P	P	P				
Uncancylus concentricus (d'Orbigny, 1835)	P	P	P	P	P		P		P
Physidae Fitzinger, 1833									
Physa aspii Holmerg, 1909*		EV							
P. loosi Holmerg, 1909*		EV				EV			
P. acuta Draparnaud, 1805+	P	P		P	P	P	P		P
Physella venustula (Gould, 1848)+					P	P	P		
Stenophysa marmorata (Guilding, 1828)	P	P	P	P	P	P			P

& Vargas, 1996) ($J = a/N$, in which a is the number of species present in two determinate basins and N is the total number of species found in both basins together) and UPGMA clustering. These analyses were run with the Multivariate Statistical Program, MVSP 3.13 (Kovach, 2006). Provinces were defined on the basis of groups of basins sharing at least 50% of their species ($J \geq 0.5$), and the basins related to these clusters, although at a lower similarity. Basins that were not included in the original analysis were later tentatively grouped with another because of hydrological or geographical features. We tentatively assigned provinces that are bounded by other countries to the political boundary because there is not enough information to extend them beyond that artificial limit.

Each province was characterized according to:

- Gastropod diversity: it was estimated using the total number of native and exotic species (Table 1), by means of the Shannon diversity index ($H = -\Sigma\ pi \ln pi$). Thus, for each prov-

TABLE 2. Values of Richness (S) and Diversity (H) obtained in each Province. Provinces: I Misionerean, II Middle Paraná, III Uruguay River, IV Lower Paraná - Río de la Plata, V Central, VI Cuyo, VII Northern Patagonia, and VIII Southern Patagonia. TZ: Transitional Zone. Hmax and Smax: maximum values of diversity and richness in each basin of each province. Hmin and Smin: minimum values of diversity and richness in each basin of each province. V: number of vulnerable species. E: number of species endemic to Argentina. EP: number of species endemic to the Province. EX: number of exotic species. IB: basins not used in the similarity analysis but tentatively included in each province.

	Provinces								
	I	II	III	IV	V	VI	VII	VIII	TZ
H	1.92	1.77	1.63	1.89	1.73	1.66	1.72	1.28	1.77
Hmax	1.92	1.67	1.66	1.9	1.82	1.75	1.75	1.33	1.73
	(b10)	(b19)	(b39)	(b33+35)	(b94)	(b55)	(b62)	(b82)	(b28)
Hmin	1.48	0.9	1.44	1.59	1.36	0.64	0.5	0.64	0.95
	(b12)	(b13)	(b43)	(b46)	(b30–31)	(b93)	(b79)	(b68)	(b84)
S	41	38	51	38	24	16	20	10	15
Smax	35	27	45	32	17	10	15	7	8
	(b10)	(b17)	(b39)	(b37)	(b48)	(b54–56)	(b63)	(b69)	(b28, 90–91)
Smin	21	13	23	15	10	3	3	3	4
	(b11)	(b25–26)	(b42)	(b46)	(b30+31)	(b93)	(b67a)	(b68)	(b60b, 61, 85)
V	9	6	14	5	3	4	6	4	1
E	7	7	12	5	4	6	5	4	3
EP	6	0	7	1	1	2	2	1	0
EX	3	2	1	2	2	3	2	0	1
Protected Areas	30	47	17	20	18	30	30	15	
Ramsar Sites	No	3	No	2	1	3	1	2	No
IB		32	38		101	58	77, 78, 61c, Part of 99	70, 71, 80, 81, Part of 99	28, 60b, 61a, 84, 85, 88, 90, 91, 98

ince and basin we did not evaluate specific diversity in the usual manner, in which each species is weighed by the relative number of individuals, but rather we considered the distribution of species among families (Rabinovich & Rapoport, 1975; Rumi et al., 2006), so that p*i* = (number of species of family *i*) / (total number of species of province or basin). Richness (S) is the total number of species for each province and for each of the basins.

- Biological value: taking into account species that are endemic (present only in one province), vulnerable (species with a limited distribution in particular areas and those not recorded since 1975), exotic and species of cattle and human sanitary importance, according to Rumi et al. (2006, 2008).
- Number of protected areas and Ramsar sites defined by The Ramsar Convention of Wetlands (Table 2).

RESULTS

From the similarity analysis, eight provinces were identified (Figs. 2–4): I, Misionerean; II, Middle Paraná; III, Uruguay River; IV, Lower Paraná – Río de la Plata; V, Central; VI, Cuyo; VII, Northern Patagonia; and VIII, Southern Patagonia.

Eight basins located in central and northwestern Argentina cluster at a low similarity index (0.23) (Fig. 2). These, together with basins with scarce records and not included in the similarity analysis (52, 87, 92, 95 and 96) and basins 83, 89 and 93 (all with three records), constitute a Transitional Zone between Provinces V and VI (Fig. 4).

Basins 102 and 93 cluster with Province VI at a low similarity value (0.32). However, they are not considered part of this province because they are very distant geographically.

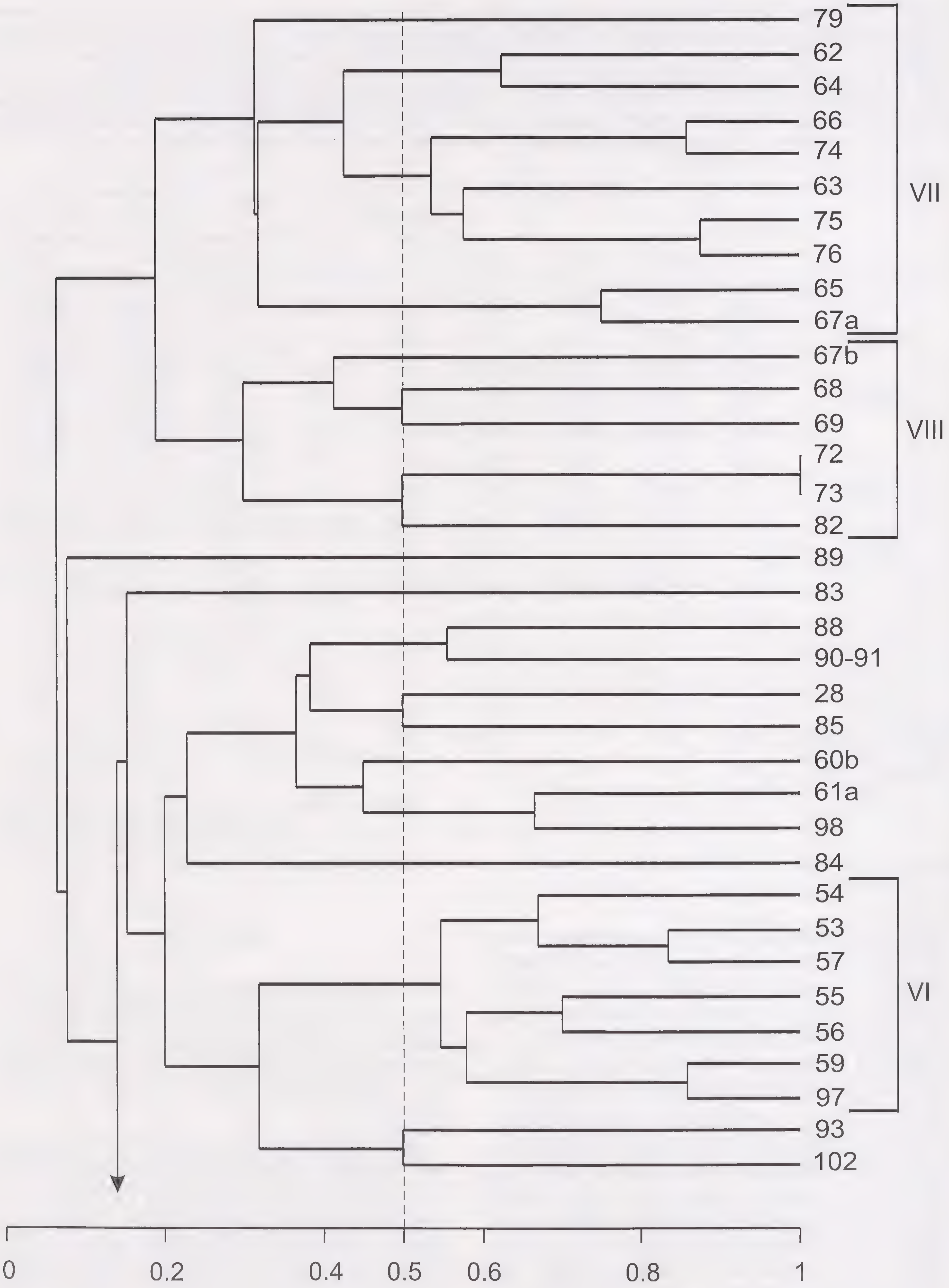

FIG. 2. Phenogram obtained from the similarity analysis among basins. Identification of provinces: VI. Cuyo; VII. Northern Patagonia; VIII. Southern Patagonia. Arrow indicates continuity with figure 3.

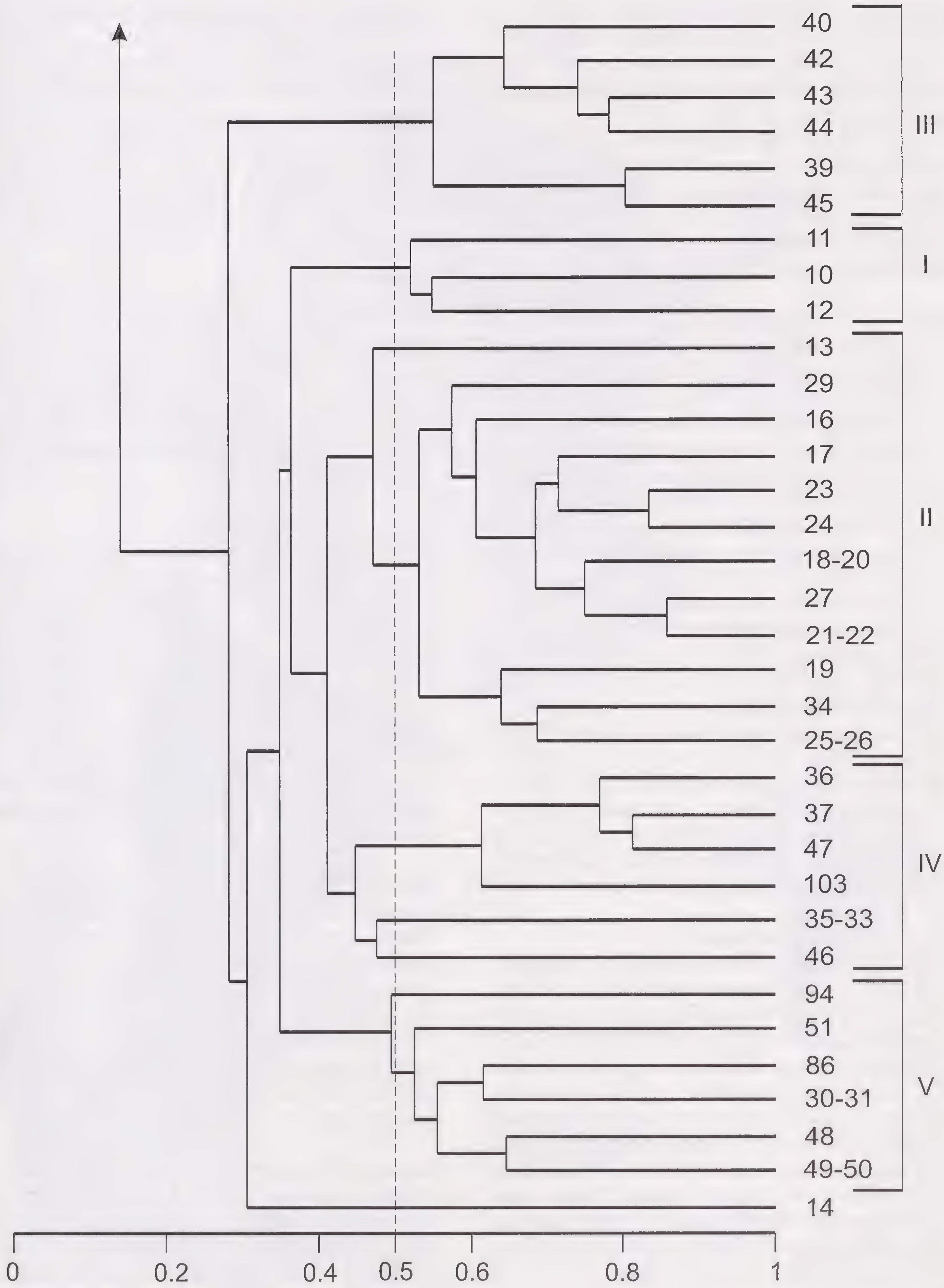

FIG. 3. Phenogram obtained from the similarity analysis among basins. Identification of provinces: I. Misionerean; II. Middle Paraná; III. Uruguay River; IV. Lower Paraná – Río de la Plata; V. Central. Arrow indicates continuity with figure 2.

Basin 102 has only six species, but two of them are endemic and considered vulnerable because they are only recorded at their type locality (*Heleobia rionegrina* and *Potamolithus valchetensis*).

Basin 14 was not assigned to any province in particular, as it clusters at a low similarity index with several provinces (Fig. 3). Perhaps, the species of this basin are more closely associated to those of basins of the neighboring countries, Bolivia and Paraguay.

Province I: Misionerean

This province shows the highest value in freshwater gastropod diversity and at the same time includes the basin with the highest diversity value for Argentina. Regarding richness, it is the second of Argentina (Table 2). The six endemic species of this province are considered vulnerable, and are found only at their type localities. *Chilina megastoma* and *C. iguazuensis* (Chilinidae) live in the Iguazú River environment, and are currently recorded frequently. *Chilina guaraniana* and *Aylacostoma* spp (Thiaridae) are endemic of the High Paraná River. Records of the former are few, while the latter has several records, but all of them before the flooding of the Yacyretá reservoir during the early 1990s. Three more species are found only in this province in Argentina but their distribution extends into neighboring countries: *Gundlachia ticaga* (Ancylidae), *Potamolithus doeringy*, and *Acrorbis petricola* (Planorbidae), the last two with a restricted distribution (Table 1).

Province II: Middle Paraná

The basin with the highest gastropod diversity of this province corresponds to part of the Cloud Forest or Yungas but the highest richness for a basin occurs in the Paraná basin (Table 2). The six vulnerable species recorded in this province have no current records (Table 1). Some of them have a restricted continuous range (*Chilina portillensis*) and others a discontinuous distribution (*Heleobia occidentalis* and *Physa loosi*). *Physa aspii* was recorded for Formosa and Salta political provinces (exact localities unknown) and has not been recorded again (Table 1).

Province III: Uruguay River

Although this is the province with the second lowest gastropod diversity, it has the highest specific richness (Table 2). This province has 20 of the 22 species of the Lithoglyphidae, which comprise almost 50% of the species present in the province. Of the 14 vulnerable species recorded in this province (Table 1), *Potamolithus concordianus* is mentioned for its type locality only and has no current records. The remaining 13 vulnerable species present a restricted discontinuous distribution. *Potamolithus tricostatus* and *P. quadratus* have no current records.

Province IV: Lower Paraná – Río de la Plata

This province receives faunal influence from Provinces II and III and therefore has mixed features. Even though *Heleobia castellanosae* and *H. isabelleana* have scarce records and there therefore vulnerable species, these are from areas where sampling was frequent.

Province V: Central

This province runs across the country diagonally from the northwest to the center-east and has numerous small and middle-sized endorrheic water courses that become salt pans during the dry season. Part of the Cloud Forest or Yungas is located in this province. The gastropod diversity index is higher and richness is almost half than in Province III (Table 2). Three vulnerable species are recorded (Table 1) and none of these have current records. *Heleobia peiranoi* is recorded only at its type locality.

Province VI: Cuyo

Gastropod diversity of this province is one of the lowest in the country (Table 2). Of the four vulnerable species in this province (Table 1), *Heleobia vianai, H. occidentalis*, and *Physa loosi* have no current records, the latter also with a restricted, discontinuous range (they are recorded also in Province II). The remaining vulnerable species, *Heleobia kuesteri*, shows restricted continuous range.

Province VII: Northern Patagonia

This province has the highest gastropod diversity of southern Argentina (Table 2). Six vulnerable species are recorded in this province (Table 1). Two of these have a restricted discontinuous range (*Chilina strebeli* and *Gondwanorbis magallanicus*), two have a restricted continuous range and no current records (*Chilina aurantia* and *C. perrieri*), one has a restricted continuous range (*Chilina*

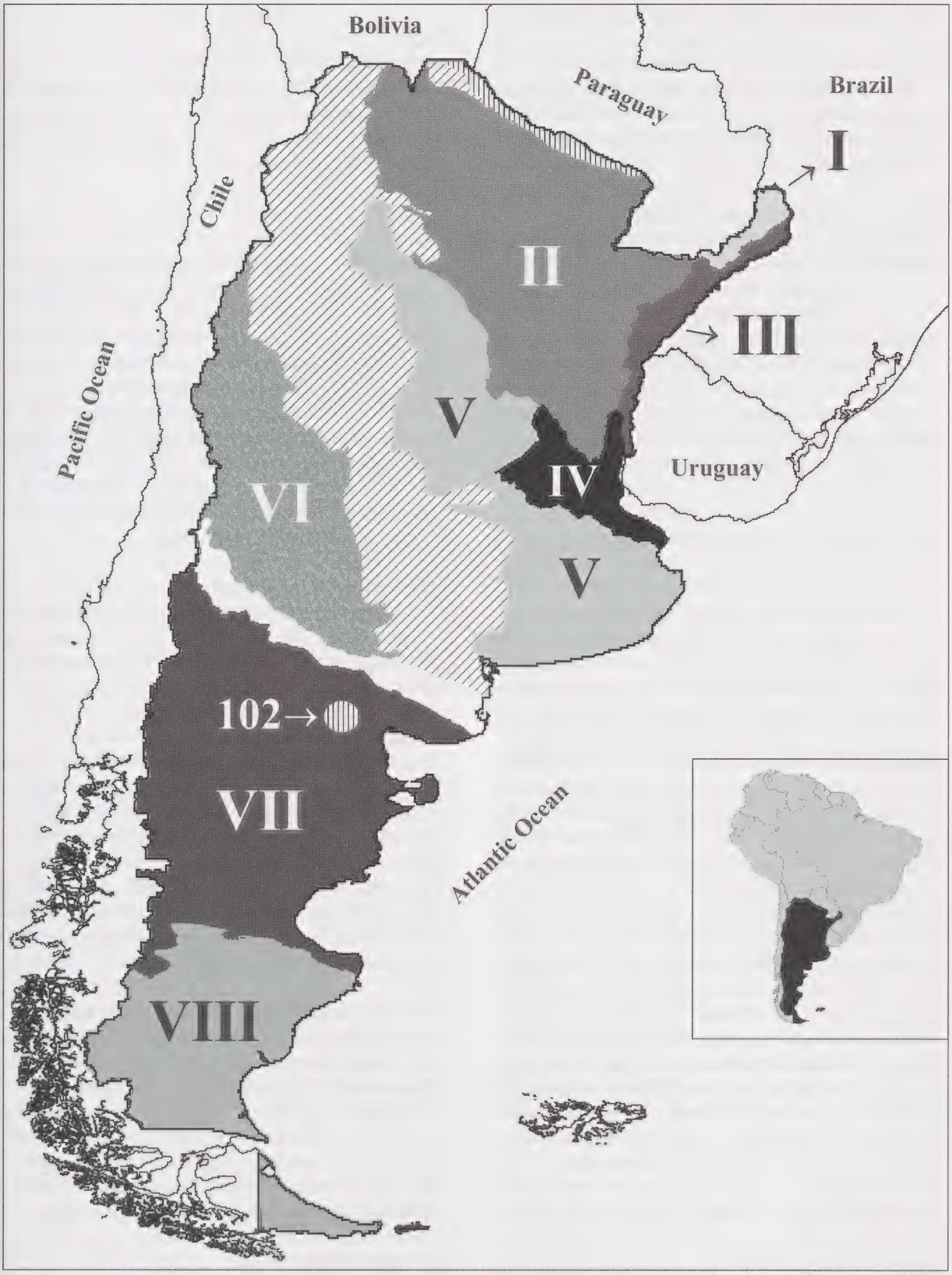

FIG. 4. Provinces identified in the map: I. Misionerean; II. Middle Paraná; III. Uruguay River; IV. Lower Paraná – Río de la Plata; V. Central; VI. Cuyo; VII. Northern Patagonia; VIII. Southern Patagonia. Diagonal patern: Transitional Zone. Vertical pattern: Basins not associated to any province. White area: Basins with no records and not associated to any province.

neuquenensis), and one is recorded only at its type locality and has no current records (*Lymnaea plicata*). *Chilina gibbosa* and *Chilina aurantia* occur only in this province in Argentina, but are also found in Chile (Castellanos & Miquel, 1980; Castellanos & Gaillard, 1981).

Province VIII: Southern Patagonia

Gastropod diversity and richness in this province are the lowest of Argentina. Gastropods are represented by a few families (Tables 1, 2). *Heleobia sublineata* is a vulnerable species endemic to this province recorded only from its type locality and has no current records. *Lymnaea pictonica* is found in Tierra del Fuego and at the southern tip of Santa Cruz; Picton Island in Chile is its type locality. There are no exotic species in this province.

Examination of the obtained phenogram reveals three large clusters. The first includes the Patagonian Provinces (VII and VIII) (Fig. 2). *Gondwanorbis magallanicus* is a species known in only two sites in Argentina, one in Province VII and the other in Province VIII. Species of Chilinidae are predominant. *Chilina strebeli* is endemic of this area. There are no species of Ampullariidae or Thiaridae.

The second large cluster is formed by Province VI and the Transitional Zone and shows greater similarity with the third cluster than with the first (Fig. 2). Species of Cochliopidae and Planorbidae are predominant over the seven families recorded (Table 1). It is worth noting the absence of Thiaridae, Glacidorbidae, and Lithoglyphidae and the presence of only one species each of Ampullariidae and Ancylidae.

The third great cluster – including provinces I to V (Fig. 3) – is the area with the highest richness values in Argentina because three different distribution patterns of freshwater gastropods overlap there. The first includes widely distributed species of Ampullariidae, Ancylidae, Physidae, Planorbidae, and Thiaridae (only in this area). Most of these species reach their southern limit in this area. The second group comprises the Chilinidae, which has a southern distribution, with its greatest richness in Patagonia, but with its northern limit in northeastern Argentina around the Tropic of Capricorn. The Lithoglyphidae constitute the third group and are almost entirely endemic to the Uruguay River basin.

DISCUSSION

Of the different biogeographic patterns mentioned in the Introduction, the pattern of freshwater gastropod provinces presented here is very similar to the ichthyological pattern (López et al., 2002):

Misionerean Province

Gastropods of this province differ from those present in streams flowing into the Uruguay River. However, according to ichthyologic classifications (Ringuelet, 1975; López et al., 2002), the whole of Misiones political province behaves as an ecoregion or province. This province is inhabited by species of *Aylacostoma* which, according to Quintana & Mercado Laczkó (1997), can be considered extinct in their natural habitat. However, Ostrowski & Quintana (2008) carried out a parasitological study on a natural population of *Aylacostoma chloroticum*.

Middle Paraná Province

It largely agrees with the wetland of Chaco (Canevari et al., 1999) and includes two ichthyologic ecoregions (4 and northern part of 2) (López et al., 2002).

Uruguay River Province

Malacologically, this province can be very well defined and is coincident with the ichthyologic proposal of López et al. (2002). No other schemes recognized this province as a single biogeographic unit. According to Bonneto (1994), the Uruguay River is part of the Del Plata basin. However, in this analysis gastropod fauna of these provinces showed a low similarity index ($J = 0.28$).

Lower Paraná – Río de la Plata Province

Gastropods of the Lower Paraná and Río de la Plata rivers receive influence of the middle Paraná and Uruguay rivers, Provinces II and III respectively. Such an influence confers the area characteristics of its own that warrant definition of a province. This does not happen in the rest of the classifications, in which this area does not conform to a separate province.

Central Province

It includes ichthyologic ecoregions 5, 7, and 8 (López et al., 2002) and coincides with the southern part of the pampas and the Chaco wetlands (Canevari et al., 1999).

Cuyo Province

It coincides with ichthyologic ecoregion 9 (López et al., 2002), while hydrographic (Bonetto, 1994) and wetland (Canevari et al., 1999) classifications include it within Patagonia. However, the fauna of gastropods of this province presents a higher similarity to the one of the Del Plata basin than to the fauna from Patagonia.

Northern and Southern Patagonia Provinces

Gastropod fauna of northern Patagonia (Province VII) differs from that of Southern Patagonia (Province VIII). Contrary to gastropods, in ichthyologic (Ringuelet, 1975; López et al., 2002), hydrographic (Bonetto, 1994), and wetland (Canevari et al., 1999) classifications, Patagonia conforms one single region. Besides, except in the ichthyologic classification of López et al. (2002), Patagonia extends to the north to include part of the Desaguadero System. In our similarity analysis, it is shown that this system defines a separate province (Cuyo) and it is more closely related to northern provinces.

Gastropod Transitional Zone

Coincides with the area of the same name defined by Bonetto (1994) based on hydrography, and it shows higher similarity to the Del Plata basins than to Patagonian basins.

On the other hand, the three great clusters of provinces of freshwater gastropods are partly coincident with the provinces proposed by Ringuelet (1975) based on ichthyology. The main difference is the conformation – for freshwater gastropods – of a central-western area (which includes Province VI and Transitional Zone), with Paraná-Plata affinities; in the ichthyologic classifications, part of the area corresponding to gastropods Province VI is included in the Patagonian Province. The cluster including provinces I to V is equivalent to the Brazilian Subregion of Bonetto (1994), and to the Paranense Dominio of Ringuelet (1975).

Analyzing the biological value, Burkart et al. (1999) showed that the highest values of biodiversity and endemism of Argentina are in the rainforests of Misiones and the Cloud Forest or Yungas. For gastropods, the highest values of diversity recorded for basins are found in the rainforest environments of Misiones (Province I) but not in the Yungas. The second most diverse place was in basins of Province IV. On the other hand, López et al. (2002) recorded the highest richness of fish species in the Middle and Lower Paraná River and in the rainforest of Misiones. In turn, Misionerean ecoregion of fish recorded a great amount of endemism. According to this classification, the Uruguay River – together with the Somuncuará System – shows the least endemism. The highest values of gastropod richness (51) occurred in Province III (Uruguay River), which also records the highest number of vulnerable (14) and endemic species (seven of the province and another five of Argentina), most of them belonging to Lithoglyphidae and to *Felliponea* spp. (Ampullariidae).

Proposals for management and protection areas at a world-wide level are established on the basis of diverse sources of information. However, invertebrates in general and gastropods in particular are not frequently used. Regrettably, most areas important for mollusk diversity have not been recognized by inclusion in the Ramsar List of Wetlands of International Importance (Strong et al., 2008). Besides, areas of highest diversity are not necessarily the same for different taxa, and some areas low biodiversity could still have an important fauna, such as the Uruguay River, with its peculiar gastropods.

Finally, it is worth noting that within the provinces it is important to identify the areas with a greater risk of degradation, either due to the construction of dams, to deforestation, or to agricultural or cattle-related activities. For example, the provinces with the greatest gastropod diversity (Provinces I and IV), according to Bertonati & Corcuera (2000), are in areas with the highest degradation level. It is important to note that Province IV is the most densely populated province of the country (more than 50%). Besides, the Uruguay River (Province III with the highest richness and number of endemic and vulnerable species of gastropods) lies within the political limits among three countries: Brazil, Uruguay and Argentina. Along its course there are at least five hydroelectric dams, and on its margin there are several industries that dispose of waste products into the river. Despite the fact that there are 17 protected areas in the province, only two of them are actually located on the Uruguay River. However, their objective is restricted to the protection of some terrestrial environments. As Suski & Cooke (2007) mention, no plans exist for the protection of aquatic environments in most of the protected areas. In addition, there are no protected areas in neighboring countries adjacent to these reserves in Argentina. Therefore, it is essential to focus the conservation plans on the basis of adequate faunal studies.

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PALEOECOLOGICAL AND PALEOBIOGEOGRAPHIC SIGNIFICANCE OF TWO NEW SPECIES OF BIVALVES IN THE PARANÁ FORMATION (LATE MIOCENE) OF ENTRE RÍOS PROVINCE, ARGENTINA

Leandro M. Pérez[1*, 2], Santiago F. Genta Iturrería[1, 3] & Miguel Griffin[1, 2]

ABSTRACT

Two new species are described based on material from the Late Miocene Paraná Formation, Entre Ríos, Argentina: ***Polymesoda muravchiki***, nov. sp., and ***Erodona doellojuradoi***, nov. sp. The bearing rocks were deposited during the Entrerriense ingression that covered part of central-northern Argentina, reaching as far north as Bolivia, Paraguay, and southern Brazil. The exposure from where the material was collected represents a rich parautochtonous assemblage – also including marine taxa – that has been assigned to the Late Miocene. Extant representatives of *Polymesoda* and *Erodona* are known to inhabit mostly brackish environments in the Caribbean region, the eastern Pacific coast of America, and Southeast Asia (in the case of *Polymesoda*), and the Atlantic coast of southern South America (*Erodona*). The presence of these bivalves in the Paraná Formation suggests that at least a marginal connection may have existed between a southern arm of the Amazonian Sea and the Paraná Sea during the Miocene. It is highly unlikely that these taxa could have migrated along the Atlantic coast of South America – contrarily to the case of the fully marine taxa – in view of their peculiar ecological requirements.

Key words: bivalves, *Polymesoda*, *Erodona*, Paraná Formation, Late Miocene, Entre Ríos.

INTRODUCTION

The marine deposits of the Paraná Formation have been known since the first half of the Nineteenth Century under such names as Entrerriense-Rionegrense, Mesopotamiense, Formación Entre Ríos, and Formación Entrerriana. Alcide d'Orbigny surveyed the area in 1827, and shortly after that it was also visited by Charles Darwin in 1833 (Darwin, 1839). D'Orbigny collected fossils at Bajada Grande (presently the city of Paraná), and he published the descriptions of his material (d'Orbigny, 1842). Darwin, while recognizing the presence of mollusks in the rocks exposed at Bajada Grande, did not provide descriptions or illustrations of any specimens, although he did comment on the geology of the area (Darwin, 1846). Later contributions towards the geological and paleontological knowledge of the area are – among others – Bravard (1858), Philippi (1893), Borchert (1901), Ameghino (1906), Ihering (1907), Frenguelli (1920, 1947), Kantor (1925), Cordini (1949), Camacho (1967a, b), Yrigoyen (1969), Iriondo (1973), Aceñolaza (1976, 2000), Aceñolaza & Sayago (1980), del Río (1990, 1991), del Río & Martínez Chiappara (1998a, b), Aceñolaza & Aceñolaza (2000), Cione et al. (2000), and Muravchik et al. (2004). The age of the Paraná Formation has been a matter of debate over the years and remains to be completely elucidated. However, most authors agree it was deposited during the Middle Miocene (Aceñolaza, 2000; Aceñolaza & Aceñolaza, 2000; del Río, 2000), or Late Miocene (Camacho, 1967a; Zabert, 1978; Cione et al., 2000).

In this paper we describe a new species of *Polymesoda* Rafinesque, 1828, and a new species of *Erodona* Bosc, 1802. The material was collected from rocks referred to the Paraná Formation exposed at La Juanita (31°52'46"S; 60°38'59"W) (Pérez et al., 2001 and Cione et al., 2008) (Fig. 1), the type locality of the new taxa described. The new species of *Polymesoda* has been reported recently from this lithostratigraphic unit by Pérez et al. (2008), and the presence of the new species of *Erodona*

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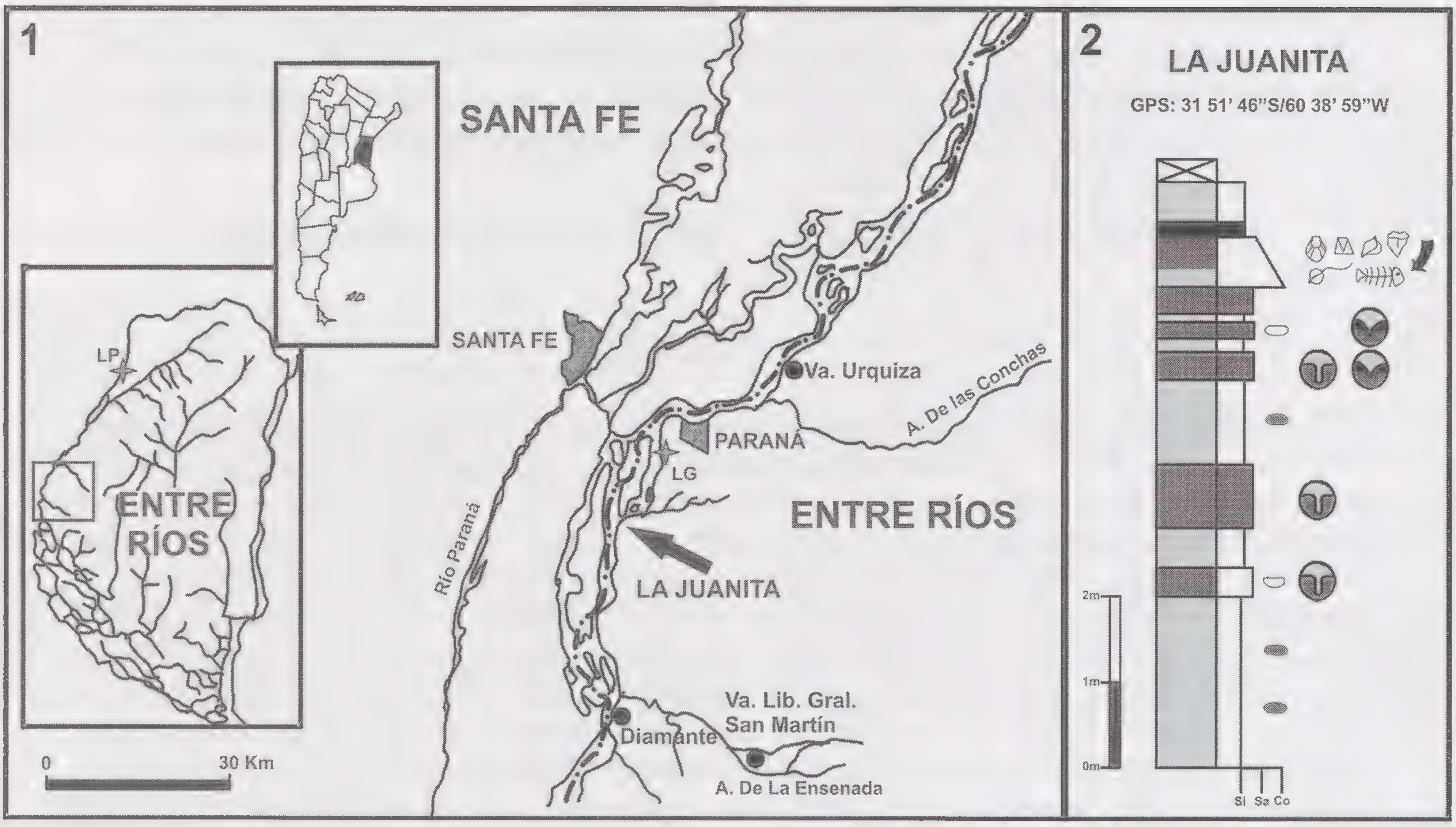
[1] División Paleozoología Invertebrados, Museo de Ciencias Naturales de La Plata, Paseo del Bosque s/n., Argentina
[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
[3] Agencia Nacional de Promoción de Ciencia y Técnica (ANPCyT)
*Corresponding author: pilosaperez@gmail.com



FIGS. 1, 2. Studied area. FIG. 1: Location map of the studied area; FIG. 2: Schematic section showing the most important lithologies exposed at "La Juanita" (LP: La Paz; LG: Los Galpones).

by Pérez et al. (2001, 2005). Aceñolaza (2004) also subsequently mentioned the presence of *Erodona* in this stratigraphic unit.

We also discuss the paleoecologial and paleobiogeographic implications of the presence of these new taxa in the Paraná Formation.

STRATIGRAPHIC SECTION

La Juanita is a locality near Aldea Brasilera village in the Department of Diamante, Entre Ríos Province. At this locality lie exposed eight meters of the Paraná Formation along the lower

TABLE 1. Measurements of specimens of ***Polymesoda muravchiki*** nov. sp. (in mm).

Collection Nº	Height	Length	Width	Observations
MAS-PI-001 HT	23.96	29.17	6.29	left valve, complete
MAS-PI-002	23.52	28.79	6.15	left valve, ventral margin broken
MAS-PI-003	14.42	16.93	3.63	left valve, complete
MAS-PI-004	15.75	18.42	4.03	left valve, anterior margin broken
MAS-PI-005	15.59	17.15	3.89	left valve, ventral margin broken
MAS-PI-006	19.55	*	4.42	left valve, posterior margin broken
MAS-PI-007	*	22.17	4.33	left valve, ventral margin broken
MAS-PI-008	23.12	28.74	6.14	right valve, complete
MAS-PI-009	18.54	21.39	4.84	right valve, complete
MAS-PI-010	16.81	19.25	4.33	right valve, complete
MAS-PI-011	17.28	19.94	4.26	right valve, complete
DMT-PI-001	24.47	28.29	6.32	left valve, complete
DMT-PI-006	22.05	28.73	6.52	right valve, complete

TABLE 2. Measurements of specimens of ***Erodona doellojuradoi*** nov. sp. (in mm).

Collection Nº	Height	Length	Width	Observations
MAS-PI-327 HT	11.19	22.06	3.91	left valve
MAS-PI-342	12.85	24.23	4.52	left valve
MAS-PI-348	12.39	21.09	4.05	left valve
MAS-PI-375	12.17	21.36	4.24	left valve
DMT-PI-145	13.17	23.63	4.49	left valve
DMT-PI-159	14.28	24.27	5.44	left valve
DMT-PI-166	8.56	15.78	3.07	left valve
MAS-PI-398	18.47	30.82	6.18	right valve
MAS-PI-399	16.24	25.63	5.08	right valve
MAS-PI-437	14.76	25.19	4.19	right valve
DMT-PI-188	15.75	26.89	4.99	right valve
DMT-PI-194	13.82	21.38	4.11	right valve
DMT-PI-217	13.72	25.06	3.65	right valve

part of the riverbank (Fig. 2). The section begins with alternating white to yellowish sands and green siltsones, very bioturbated by *Ophiomorpha* isp., followed by an erosional bed of lenticular geometry and laterally continuous for about 100 m. This bed shows normal gradation with a sabulitic matrix at the bottom and grading upwards to a sandy bed. Towards the middle part, the sandy matrix is strongly cemented by carbonate. The thickness of this bed reaches about 50 cm, and it contains the remains of the *Polymesoda* and *Erodona* described herein. These are associated to a fairly rich fauna of other invertebrates and vertebrates. The base of the section is usually covered by debris fallen from the adjoining cliff or by river during floods. The top is covered by vegetation.

MATERIALS AND METHODS

The described specimens were recovered after washing shells and sediment from the shell bed, using a dilute solution of H_2O_2. Valve measurements were taken using a 0–150 mm digital caliper. Specimens were observed under light microscopy and photographed using a digital camera. Measurements (Tables 1, 2) are taken according to Stanley (1970). Dimensions are in millimeters. Abbreviations used are L = length; H = height; W = width.

Acronyms

All examined material is housed in the collection of the División Paleozoología Invertebrados at the Museo de La Plata (MLP), the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN), the Museo de Ciencias Naturales y Antropológicas “Prof. Antonio Serrano”, Paraná, Entre Ríos (MAS), and the Centro de Investigaciones Científicas y de Transferencia Tecnológica a la Producción (CICYTTP), CONICET, Diamante, Entre Ríos (DMT).

SYSTEMATIC PALEONTOLOGY

Class BIVALVIA
Order VENEROIDA
Superfamily CORBICULOIDEA
Family CYRENIDAE
Genus *Polymesoda*, Rafinesque, 1828
Type Species *Cyclas caroliniana* Bosc, 1802,
by original designation

Polymesoda muravchiki, nov. sp.
FIGS. 3–10

Material: Thirteen specimens: eight left valves, of which three are complete and five broken; five complete right valves.

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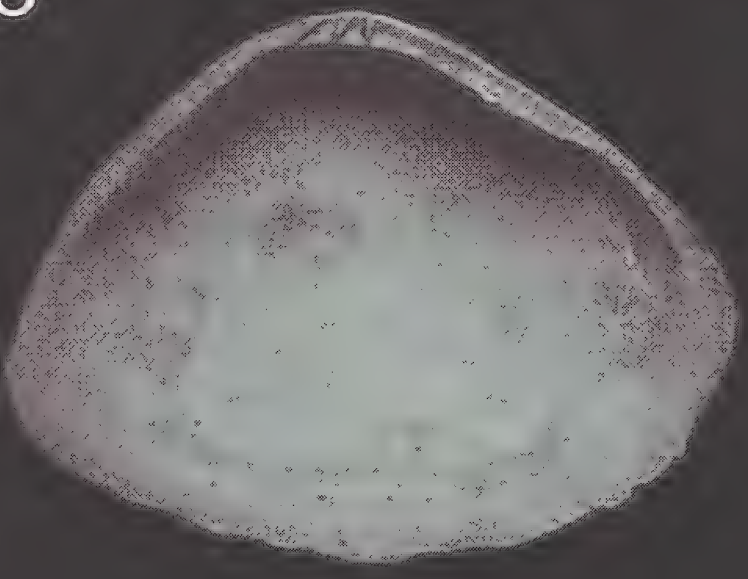

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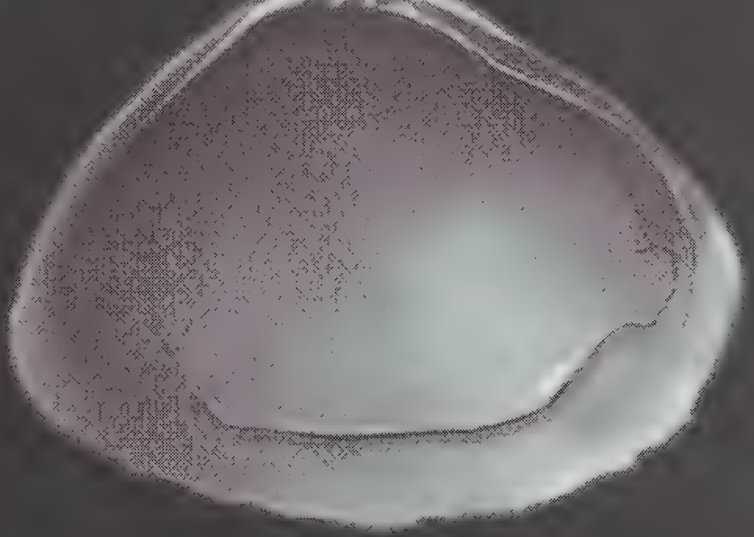

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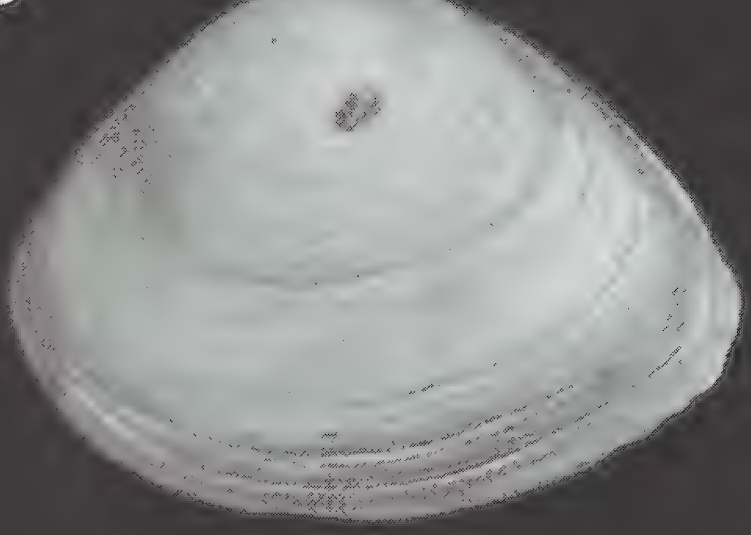

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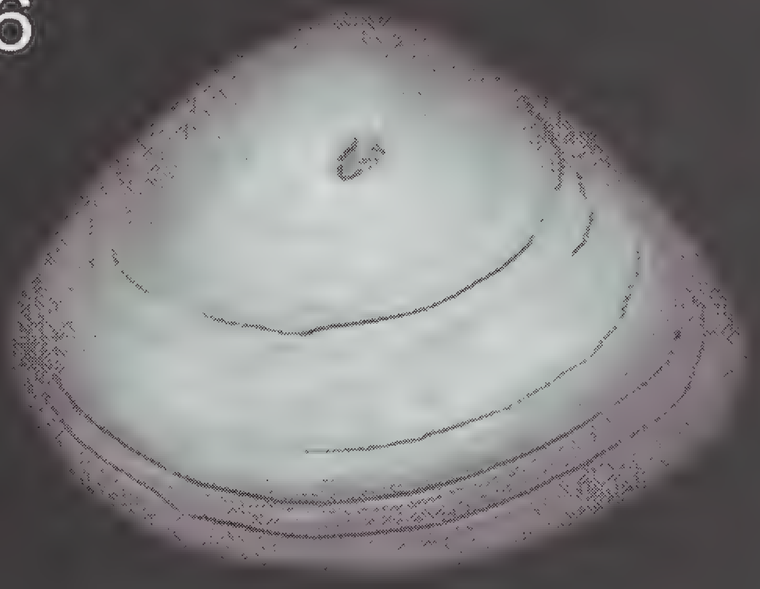

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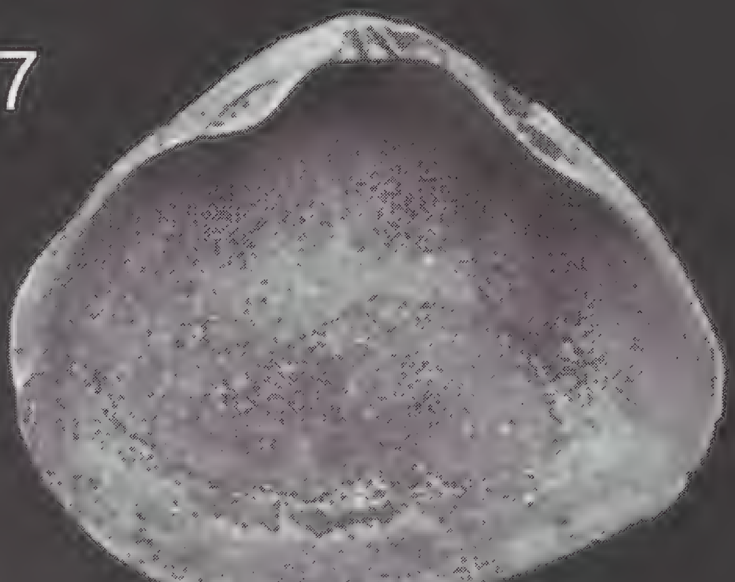

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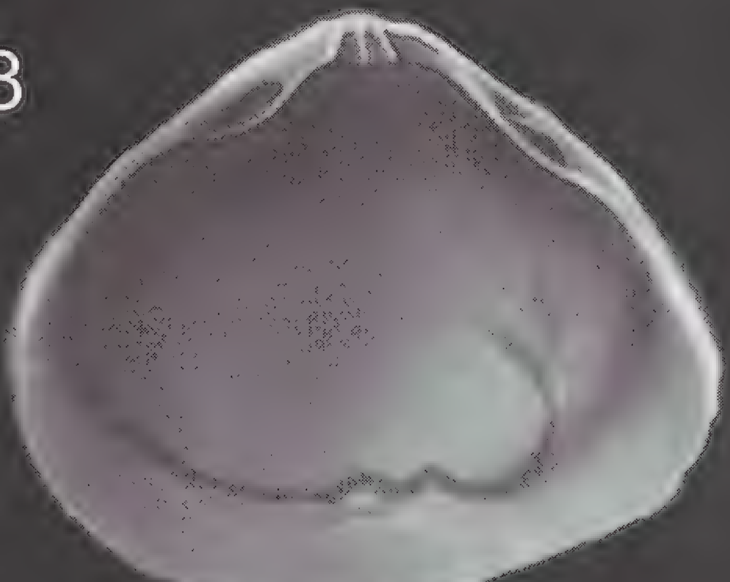

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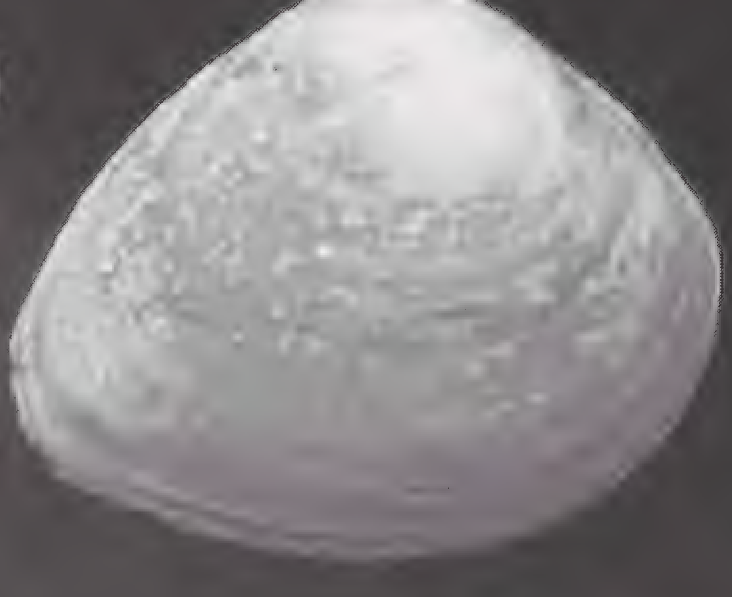

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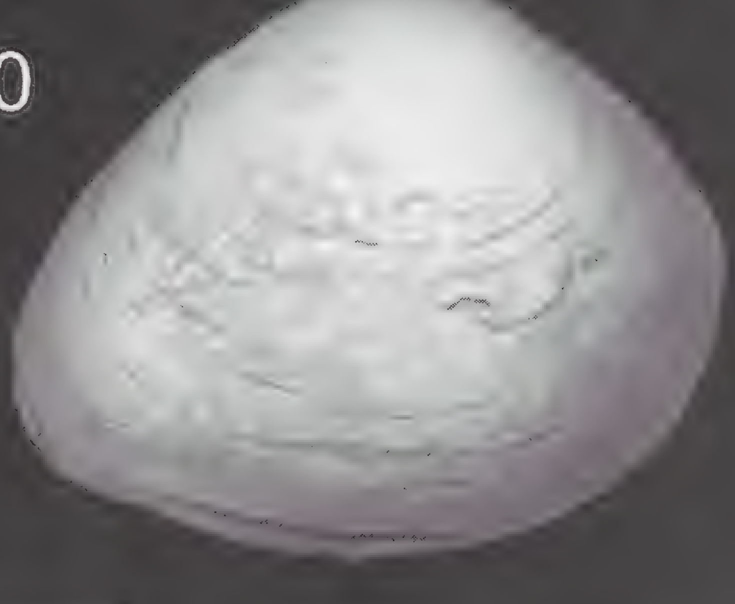

Type Material: Holotype, MAS-PI-001 a left valve; all other specimens are paratypes, MAS-002-011, DMT-PI-001and DMT-PI-006.

Type Locality: La Juanita (31°52'46"S, 60°38'59"W), Aldea Brasilera, Diamante Department, Entre Ríos Province, Argentina.

Occurrence: This new species is found only within the Paraná Formation at La Juanita and is thus far represented by only 13 valves.

Etymology: After Martín Muravchik, who was instrumental in collecting the specimens described.

Diagnosis: *Polymesoda* with equilateral and triangular shells with convex ventral margin, laterally compressed, height 82% of length; umbo low, non-protruding, mesogyrous; ornamentation of weak commarginal lines; left valve anterior and middle cardinal teeth short, small, lamellar, subparallel and vertical; left valve posterior cardinal tooth flattened and defining a small cavity parallel to posterodorsal margin; nymphae narrow.

Description: Shell small-sized for genus (17 to 29 mm long), triangular, nearly as high as long; shell laterally compressed; umbones mesogyrous located at mid-length of valves; anterior dorsal margin moderately convex; posterodorsal margin straight to slightly convex, descending steeply to join regularly convex ventral margin; posterior end slightly truncate; ornamentation of very delicate commarginal lines; adductor muscle scars deeply impressed, similar in size; left valve cardinal teeth three, the anterior and the middle teeth sharp and lamellar and the posterior tooth very weak, barely differentiated from the narrow hinge plate that separates it from the nymph; right valve cardinal teeth three, lamellar and weak (Figs. 7, 8); left valve anterior and posterior lateral teeth elongate, smooth, adjacent to inner surface of shell; left valve anterior lateral stronger and half as long as posterior tooth; right valve with two anterior parallel lateral teeth and two parallel posterior teeth; inner lateral teeth lamellar and much stronger than external ones, which are almost obsolete and barely differentiated from the hinge plate; nymph short and narrow, widening posteriorly and almost reaching proximal end of posterior lateral tooth.

Measurements: HT (MAS-PI-001): L = 29.17; H = 23. 96; W = 6. 29.

Remarks: The taxonomic status of this species requires assessment of previous records of Corbiculidae in the region, especially in the Paraná area, as some of the nominal species included in this group may be superficially similar to our material and may lead to confusion in its correct taxonomic placement and affinities.

The earliest species recorded that shows some resemblance to ***P. muravchiki***, nov. sp., is *Cyrena salobris* Ihering, 1907 (pp. 413–414, pl. 16, fig.109a, b). Ihering based this species on three specimens (MACN-HT 336) collected by Carlos Ameghino at Sierra Laziar, Santa Cruz Province, Argentina. Ihering placed this species in the subgenus *Pseudocyrena* Bourguignat, 1854, but the shell characters described by Ihering and examined by us in his original material suggest that it fits better in *Polymesoda*, as it shares the same hinge with three cardinal teeth, smooth anterior and posterior lateral teeth, short nymph, and very small pointed pallial sinus. It was later placed doubtfully in *Neocorbicula* by Parodiz (1969: 95–96, pl. 10, figs. 14, 15), because of the "general similarities". Ihering (1907) stated that he had three specimens, but later Ihering (1914: 41) mistakenly mentioned only two. Parodiz (1969: 95) stated that the "type" was a worn shell housed in the MACN, although he did not provide further information. However, he referred to the "type" as the figured specimen in his plate 10, figs. 14, 15 (Parodiz, 1969), which is a valid lectotype designation under the provisions of ICZN Article 74.5, as stated late by Parodiz (1996: 266). Del Río (1994: 109–110, text-fig. 2a, b) described

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FIGS. 3–10. ***Polymesoda muravchiki*** nov. sp., scale = 2.5 cm. FIGS. 3, 4: Left valve MAS-PI-001 HT, internal view (Fig. 3: photograph, Fig. 4: drawing); FIGS. 5, 6: Left valve MAS-PI-001 HT, external view (Fig. 5: photograph, Fig. 6: drawing); FIGS. 7, 8: Right valve MAS-PI-008, internal view (Fig. 7: photograph, Fig. 8: drawing); FIGS. 9, 10: Right valve MAS-PI-008, external view (Fig. 9: photograph, Fig. 10: drawing).

Polymesoda (*Egeta*) *salobris* (Ihering, 1907) and stated that Ihering's figured specimen (Ihering, 1907: pl. 16, fig.109a, b) was the holotype. Under the name of *Polymesoda* (*Egeta*) *salobris* (Ihering, 1907), del Río (1994) also included material collected from the Puerto Madryn Formation at Fondeadero Ninfas, Chubut, northern Patagonia. This material (CPBA 14170/73; del Río & Martínez Chiappara, 1998b: pl. 9, figs. 1–3) is clearly different from Ihering's species, as the much larger shells have a more elongate, subtriangular shape and stronger commarginal lines; in addition the middle cardinal tooth is longer and the lateral cardinal teeth are shorter. All the type specimens of *Polymesoda salobris* (Ihering, 1907) are much smaller than our specimens. They are also more rounded and somewhat more elongate and wider than the new species, which is larger and with a more triangular shape than Ihering's. The cardinal and lateral teeth in the new species are weaker, as also are the umbones and the commarginal lines.

A second species apparently belonging in the Cyrenidae is *Corbicula* (*Corbiculella*) *tenuis* Ihering, 1907 (pp. 468–469, pl. 18, fig. 130a, b), based on material embedded in the matrix within *Diplodon fraus*, a freshwater bivalve collected by Bravard at Paraná, but probably from continental beds overlying the Paraná Formation. This species was based on fragmentary material now missing from Ihering's collection. The description is very general and the illustration is very poor – shedding no light on its true identity. Ihering stated that he had no complete specimen of this species, which had thin and fragile valves. He also stated that these were poorly inflated ("*peu ventrue*") and more or less oval-shaped, with a non-prominent umbo. In these and other characters, such as the obliquely ascending anterior and posterior margins, smooth outer surface, and general outline, this species may be confused with ***P. muravchiki***, especially as the drawing provided by Ihering (1907: pl. 18, fig. 130a, b) is very poor and no distinctive feature clearly depicted. However, in Ihering's description, he stated that the median cardinal tooth of the left valve is bifid, and that it has two anterior lateral teeth. These features – together with the purported lack of pallial sinus – are enough to clearly distinguish it from ***P. muravchiki***. Ihering proposed the new subgenus *Corbiculella* for *Corbicula tenuis*. Parodiz (1969: pp. 98–99, pl. 10, figs. 12, 13) reassigned it to *Neocorbicula*, while recognizing *Corbiculella* as a subgenus. Unfortunately, he did not provide any further description or illustration of new material, basing his comments on Ihering (1907). According to Parodiz (1969: 98), the specimen illustrated by Ihering represents two different left valves, but the poor drawings are inconclusive on this issue, although they do in fact appear to belong to the same specimen. Parodiz dismissed Ihering's statement on the lack of pallial sinus because it could not be seen on the illustrated syntype, but Ihering had additional specimens. According to Ihering (1914: 41) he had four specimens from Paraná ("cotypos", from the Bravard Collection; MACN-PI #337, according to Parodiz, 1996) and additional material from La Paz collected by S. Roth (several specimens embedded in rock, the only ones presently remaining in Ihering's collection). Therefore, it seems reasonable that he would have recorded the presence of a pallial sinus in at least some of the specimens, as it appears to be a character that he would not have missed if present; he was probably sure enough of its absence to mention that fact in his description. In any event, even if superficially similar to the new species described herein, the two taxa are different and belong in different genera, in which case we would agree with Parodiz (1969) in placing it in *Corbiculella*. A decision on whether this is a subgenus of *Neocorbicula* or a different, unrelated group must wait until more data on the type species becomes available.

Polymesoda muravchiki easily fits in *Polymesoda* because of its shape and its characteristic hinge with smooth teeth. Previous inclusions of material referable to *Polymesoda* in any of the different subgeneric divisions (Keen & Casey, in: Moore, 1969) appear to be in need of revision and must wait until a general systematic study of the group is undertaken.

From *Polymesoda caroliniana* (Bosc, 1802), the type species of the genus, it can be distinguished by its much lower and inconspicuous umbos and its weaker lateral teeth (Van der Schalie, 1933). The Recent species also has a higher shell and the pallial sinus is deeper, more pointed and not directed as dorsally as in ***P. muravchiki***.

In these characters, it resembles more *Polymesoda arctata* (Deshayes, 1854), also Recent from the Caribbean coast of South and Central America. This species has a higher and more prominent umbo, and the ornamentation is far more conspicuous. The two species share the same type of pallial sinus, but the teeth are much stronger in the extant one.

A recent species that more closely resembles the fossil one described herein is *Polymesoda floridana* (Conrad, 1846), from the Caribbean coast of North America. Material examined by us (MLP-I-9846) shows that the shell outline and hinge are very similar to that of ***P. muravchiki***, but the lateral teeth are stronger in the living species. It also shows a more clearly defined posteroventral ridge.

Presently, the most austral species of this genus in South America is *Polymesoda notabilis* (Deshayes, 1855), which inhabits the Pacific coast near Tumbes, Peru. According to material of this species we examined (MACN-I-29222), it differs from ***P. muravchiki*** in its larger, inequilateral valves, and its lateral teeth are smaller and sharply pointed.

In the Argentine geological literature there are many references to *Neocorbicula stelzneri* Parodiz, 1969 (pp. 93–94 pl.11, figs. 2, 3), from localities in the northwestern area of the country and within rocks generally correlated with the Paraná Formation. However, the identity of the material referred to it remains uncertain, as most specimens housed in museums are only very badly preserved casts in which no interior characters are visible. Thus, any comparison with it is meaningless until specimens with visible hinges and pallial lines become available.

Order MYOIDA
Superfamily MYOIDEA
Family ERODONIDAE
Genus *Erodona* Bosc, 1802
Type Species *Erodona mactroides* Bosc, 1802; subsequent designation by Dall, 1898

Erodona doellojuradoi nov. sp.
FIGS. 11–18

?*A. occidentalis* Ameghino, 1891: 89 (*nomen nudum*).
?*Corbula mactroides* Daud. var. *paranensis* Doello-Jurado (n. var.) – Frenguelli, 1920: 99 (*nomen nudum*).

Material: Two hundred and sixty specimens, 100 left valves and 160 right valves.

Type Material: MAS-PI-327, a left valve, is designated holotype (HT). Paratypes, MAS-PI-328/386, and DMT-PI-143/182, a left valve; MAS-PI-387/486 and DMT-PI-183/242, a right valve.

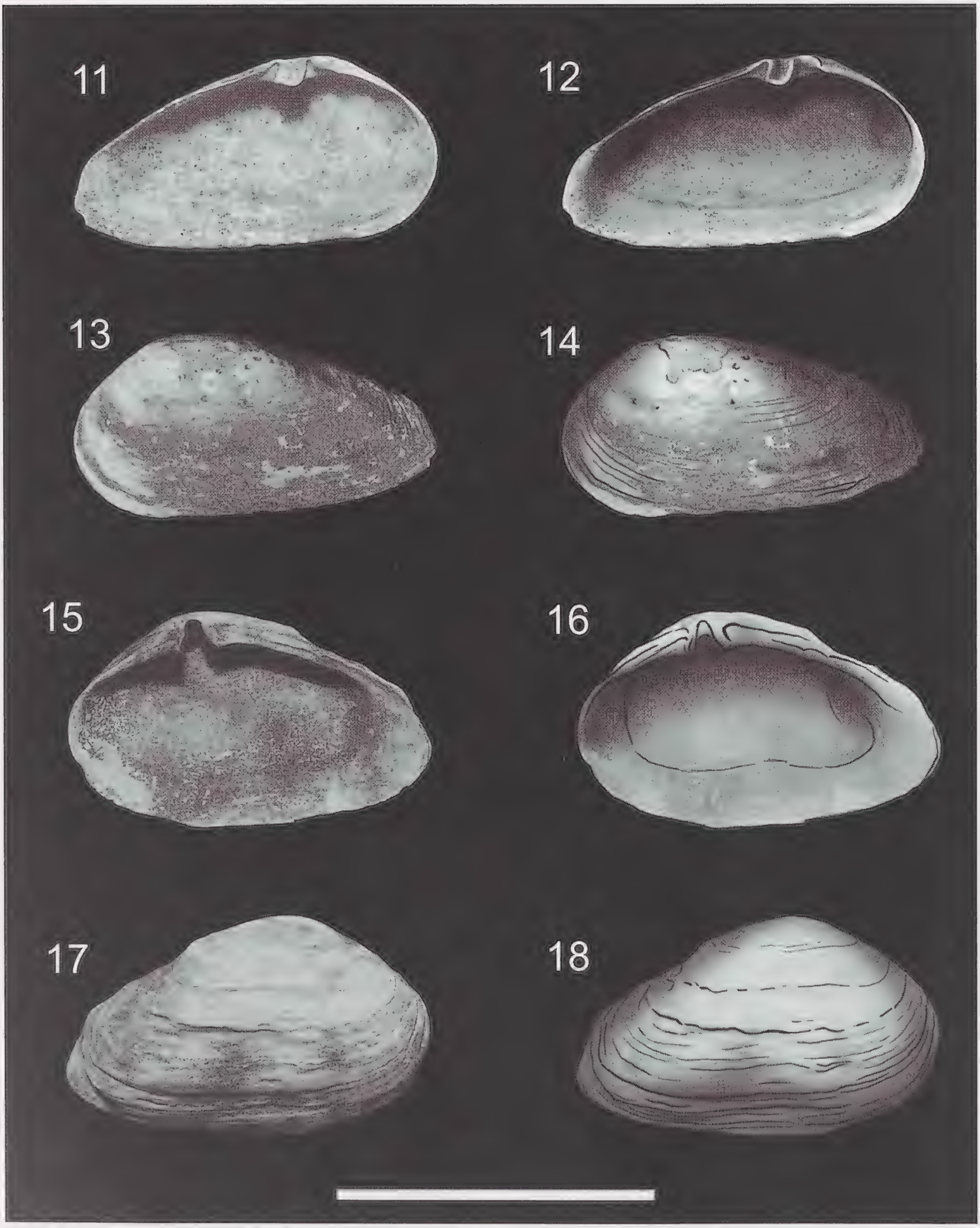
Type Locality: La Juanita (31°52'46"S, 60°38'59"W), near Aldea Brasilera, department of Diamante, Entre Ríos Province, Argentina.

Etymology: Honoring Martín Doello-Jurado, who recognized that this was a new taxon in 1920 (Frenguelli, 1920: 96).

Occurrence: Most specimens were found within the Paraná Formation at La Juanita. One specimen was found embedded in the matrix surrounding a specimen of *Anadara bonplandiana* (MLP 7908) labelled as from La Paz, Entre Ríos. A second specimen, a right valve (MLP 1819), comes from the Arroyo Los Galpones, nearby Paraná.

Diagnosis: Shell corbuliform, strongly inequivalve; umbones not prominent, placed at 0.38 of total length from anterior end; right valve with very shallow sulcus running from umbo to ventral margin; subtriangular asymmetric chondrophore pointing posteriorly, with concavity facing frontwards; posterior half of chondrophore almost perpendicular to commissure plane; anterior edge of chondrophore inner surface with narrow, weak but conspicuous groove.

Description: Shells thin, corbuliform, strongly inequivalve, inequilateral; commissure not lying in a plane; length between 9.65 and 31.31 mm and height between 6.14 and 15.89 mm (LV); length between 10.27 and 30.82 mm and height between 7.63 and 19.64 mm (RV). Right valve (Figs. 15, 16) umbo more prominent than that of left valve, placed at 0.38 of total length from anterior end; distinct umbonal ridge running nearly parallel to posterior margin, rendering posterior end truncate; very shallow sulcus running from umbo to ventral margin, originating an indistinct shallow depressed area on shell surface; left valve smaller, shallower than right valve; left valve umbones very indistinct, as well as shallow medial sulcus; hinge plate narrow, long; right valve hinge with two very weak cardinal teeth, diverging from umbo, border-



FIGS. 11–18. ***Erodona doellojuradoi*** nov. sp., scale = 2.5 cm. FIGS. 11, 12: Left valve MAS-PI-327 HT, internal view (Fig. 11: photograph, Fig. 12: drawing); FIGS. 13, 14: Left valve MAS-PI-327 HT, external view (Fig.13: photograph, Fig. 14: drawing); FIGS. 15, 16: Right valve MAS-PI-398, internal view (Fig. 15: photograph, Fig. 16: drawing); FIGS. 17, 18: Right valve MAS-PI-398, external view (Fig. 17: photograph, Fig. 18: drawing).

ing a deep triangular resilifer that is generally directed posteriorly; anterior cardinal tooth of right valve larger than lamellar posterior tooth; left valve hinge with a short, projecting chondrophore surrounded by sockets for the corresponding teeth of right valve; anterior socket larger, separated from chondrophore by a weak, low ridge running along posterior edge of chondrophore; a similar ridge, much sharper, separates the small anterior tooth socket from the chondrophore; shell isomyarian, with anterior adductor muscle scar reniform and slightly larger than subcircular posterior scar; anterior pedal retractor muscle scar small, subcircular, placed at mid-distance between umbo and anterior adductor muscle scar; pallial line well marked, with a very shallow, indistinct pallial sinus adjacent to posterior adductor muscle scar; external surface of shell smooth, with weak and regularly spaced commarginal growth lines.

Measurements: HT (MAS-PI-327): L = 22.06; H = 11.19; W = 3.91. Measured paratypes: MAS-PI-342/348/375 and DMT-PI-145/159/166, left valves; MAS-PI-398/399/437 and DMT-PI-188/194/217, right valves.

Remarks: Previous mentions in the literature that may be referable to this species were published by Frenguelli (1920). This author indicated the presence of isolated valves of "*Corbula mactroides* Daud. (= *Azara labiata* d'Orb.)", and he pointed out that it was previously unknown from Tertiary beds in Entre Ríos, and an important member of the fauna. On the other hand, in a footnote to his comment he quoted Doello-Jurado (*in littera*) who stated that this *Corbula* could not be identified with the extant *Azara labiata*, representing a variety or an immediate precursor to *Corbula mactroides,* presently living in the Río de La Plata estuary. He named it *Corbula mactroides* Daud. var. *paranensis*, n. var. (Frenguelli, 1920: 99) [*nomen nudum*]. *Erodona* was subsequently mentioned without illustration nor indication of species by Aceñolaza (2004: 27).

Erodona is represented in South America by its type species, *Erodona mactroides* Bosc, 1802, which inhabits mainly low salinity environments along the Atlantic coast of South America from the Lagoa dos Patos, Rio Grande do Su, Brazil (Rosa & Bemvenuti, 2006), to Punta Rasa, Buenos Aires Province, Argentina. This species was first described based on material collected from an unknown locality ("probably South Atlantic Ocean" according to Lamy, 1941) that has not been identified yet, but is probably the estuary of the River Plate (Maton, 1809). Representatives of *Erodona* have been mentioned from late Eocene rocks (Keen, in: Moore, 1969), but these references have not been confirmed and possibly belong in other genera. It has been recorded with certainty in Pleistocene and Holocene deposits of the same region (Wahnish, 1942; Camacho, 1967b; Aguirre, 1988, 1994; Farinati et al., 2006).

A few years later, d'Orbigny (1839: 573, pl. 72, fig. 22; 1842: 161) described *Azara labiata*. His material was collected from the Pampéen, Buenos Aires Province, near the town of San Pedro, about 60 km upstream from the mouth of the Paraná River.

The species was revised by Carcelles (1941), and he commented on its variability, affinities, and geographic range. He concluded that this species, although often mentioned in the literature since it was first described, had been erroneously identified. According to him, this was so in part because of the variability it shows and because of the poor knowledge then available on this variation. Most of the references in the literature are based on material collected by geologists carrying out field work often in connection with building sites in areas surrounding the city of Buenos Aires. It was also mentioned from localities along the Atlantic coast from São Paulo down to Punta Alta, near the city of Bahía Blanca, Buenos Aires Province (Farinati et al., 2006). References of this species also exist from Gualeguaychú, along the coast of the Uruguay River, about 250 km north of Buenos Aires, and from Atalaya and Río Salado, both located south of La Plata along the coast of Buenos Aires Province (Carcelles, 1941). Most of the material mentioned by Carcelles and previous authors is missing and thus such identifications could not be confirmed. Therefore, additional material housed in museums in Argentina (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires and Museo de La Plata "Francisco Pascasio Moreno", La Plata) was analyzed. Such an analysis suggests that Carcelles´s ideas on the variability of this species were generally right. This is true at least for all the material collected from units now referred to the "Pampeano" and "Post-Pampeano", exposed between San Pedro and Bahía Blanca in Buenos Aires Province (Camacho, 1967b; Aguirre, 1988). The case is different with those references mentioned by Carcelles in which the material surely comes from older beds, for example, the specimens mentioned by Doering (1882: 405) from the "Araucano" of the Santa María Valley in Catamarca, western Argentina, which Ameghino (1891: 89) referred to as *A. occidentalis*. No description or illustration was provided, and therefore it should be considered a *nomen nudum*. However, further research in western Argentina proved the existence there of marginal marine rocks referred to the Entrerriense transgression (Herbst et al., 2000). Most likely, the material mentioned by Doering may belong in this new taxon.

A second doubtful reference of this species is by Szajnocha (1888), who reported it from Magallanes and Skyring Water, in southernmost Chile. However, this record appears to be referable to *Corbula*, as suggested by Ihering (1907) and Carcelles (1941).

The only Recent species of this genus, *Erodona mactroides*, is known to occur profusely in the Río de La Plata estuary, where if forms relatively large shell accumulations. It has also been reported from Lagoa dos Patos in southern Brazil (Bemvenuti et al., 1978; Geraldi, 2002; Rosa & Bemvenuti, 2006), Uruguay (Maldonado, Punta del Este, Playa Capurro, Montevideo, and Colonia del Sacramento), and in Argentina (Buenos Aires, Río Matanza, Río Santiago, Atalaya, Magdalena, Punta Indio, Punta Piedras, and Bahía de Samborombón). All these localities lie in the temperate areas of the Atlantic coast of South America, and are all in brackish environments in which the salinity ranges between 7 g/l and 20 g/l (Ríos, 1975; 1985). According to Da Costa (1971), it is more frequent in pre-mixohaline (8–15 g/l) and mixohaline (3–9 g/l) zones, becoming uncommon in pre-limnic zones in Lagoa dos Patos, southern Brazil. Sprechman (1978) considered it a genuine brackish species from mesohaline environments (3–18 g/l). Da Costa (1971) suggested that *Erodona mactroides* is a typically mixohaline taxon showing a distribution adapted to the environment. It has been mentioned neither from fully marine environments in the area, nor from clearly freshwater environments. The recorded stratigraphic range of the genus was Eocene? - Recent. However, the early Cenozoic records from South America seem to be incorrect, as the species actually involved can be now placed in other genera that, while similar to *Erodona*, are quite different. Aguirre (1994) summarized a synonymy list for *Erodona mactroides* and at the same time provided data on the distribution of this species in Quaternary deposits in Argentina.

Nuttall (1990) revised the faunas of non-marine mollusks from Cenozoic inland basins from northwestern South America. In this work, he stated that *Erodona* shows a very different hinge from that of *Pachydon* Gabb, 1869 (type species *Pachydon obliquus* Gabb, 1869), which is clearly visible in the specimens of several species figured by him. The hinge in *Pachydon* carries a strong peg-like cardinal tooth in the right valve, which is absent in *Erodona*, in which the anterior tooth, adjacent to the resilifer, is weak and narrowly blade-like. The chondrophore in the left valve of the southern genus is also different, as it lies at an angle to the commissure plane and its inner surface faces posteriorly.

A genus that in some ways is similar to *Erodona* is *Ostomya* Conrad, 1874 (type species *Ostomya papyria* Conrad, 1874). This genus, placed by Keen (in: Moore, 1969) in the Lyonsiidae Fischer, 1887, was allocated by Nuttall (1990) to the Corbulidae Lamarck, 1818. The hinge of the type species resembles the hinge of *Erodona*. In *Ostomya*, the right valve carries a narrow blade-like tooth very similar to that observed in *Erodona*. It fits in a correspondingly narrow socket in the left valve hinge. The resilifer is also inclined, although in *Erodona* it is proportionally better developed, and in the left valve it rests in a chondrophore that is facing posteriorly. As in *Ostomya*, the resilifer in the right valve is deeply sunken but it is far more strongly buttressed. The nymph in the left valve is entirely missing in *Erodona*, a feature that separates it from Conrad's genus. Also, the shells of *Ostomya* are thinner and more ovoid in shape than those generally observed in *Erodona*.

Worth mentioning is the case of *Anisothyris iquitensis* de Greve (1938), from Tertiary beds near Iquitos, Peru. Bristow & Parodiz (1982) suggested that this species was closer to *Erodona* than to *Anisothyris*, where it had been originally placed by de Greve (1938).

According to them, the main difference with *Erodona* was the posterior end of the shell, which is concave instead of convex, and has an obsolete filiform ligament along the marginal line at both sides of the umbo. They also stated that it is possible that *A. iquitensis* is a transitional form or a subgeneric intermediate between *Erodona-Anisothyris*, and related to the true marine *Corbula* (*Caryocorbula*). However, Nuttall (1990) believed that it belonged in *Pachydon* and allocated it to that genus. He also stated that a further specimen from the Cuenca Basin, Ecuador, discussed by Bristow & Parodiz (1982: 31) belonged in *Corbicula* (*Cyanocyclas*) *cojitamboensis* Palmer, in Liddle & Palmer (1941).

Erodona doellojuradoi can be separated from the type species by a number of characters. The fossil species has generally slightly larger shells than *Erodona mactroides*. The shell outline is also different, with a straighter ventral margin, which renders the shell generally more elongate, with H/L ratios of 0.56 (LV) and 0.61 (RV), in contrast to those of *Erodona mactroides* (0.64, LV and 0.66, RV; pers. obs.). The posterordorsal margin is very gently convex, whereas in *E. mactroides* it is straight or slightly concave. This feature renders the umbos less prominent than in the extant species and the posterior part of the shell is more rostrate. The hinge also shows differences, particularly in the development of the hinge plate, which is lower, narrower and more slender than in the type species. The cardinal teeth of the right valve are also more delicate. The chondrophore, while smaller, is clearly asymmetrical, as its posterior ridge is far more developed and higher than the anterior one. This assymetry gives the chondrophore its "anteriorly inclined" appearance. On the other hand, the chondrophore of the living species is larger, and the ridges are not much different from each other. Thus the chondrophore appears to lie more or less parallel to the commissure plane.

As in *Erodona mactroides*, ***Erodona doellojuradoi*** has a weak groove that borders the posterior edge of the chondrophore, but this groove is better defined in the extant species. Growth lines in ***E. doellojuradoi*** are weaker and more regularly placed than in *Erodona mactroides*, rendering the shell surface smoother. The shell is also thinner in the fossil species. Also, the shells are slightly less inflated in the fossil species (W/H = 0.34, LV, and 0.30, RV) than in the extant one (W/H = 0.37, LV, and 0.36, RV), a trait that remains consistent throughout ontogeny. While the shape of the shells from different populations of *Erodona mactroides* appears to be at least partly dependent on such environmental factors as salinity, temperature, the influence of these in the fossil populations remains yet to be ascertained.

Paleoenvironmental & Biogeographic Significance

The genus *Polymesoda* Rafinesque, 1828 (Mollusca: Bivalvia: Corbiculidae), is presently represented in America by several species living mainly in the Caribbean region. It is also known from the eastern Pacific (Gulf of California to northern Peru) and the Indo-Pacific (Woodward, 1964; Morton, 1976). Species of this genus inhabit silty substrata in brackish estuaries, mangroves, and near river mouths (Castaing et al., 1981; Marelli, 1990; García et al., 1994; De La Hoz Aristizábal, 2005, 2008). The genus is also known from Eocene to Recent deposits in North and Central America (Duobinis-Gray & Hackney, 1982; Rueda & Urban, 1998; Sarcos & Botero, 2005), and the Recent southernmost range is probably northern Peru (pers. obs., LMP); in Argentina the genus was recorded in the Pliocene (?) of Sierra Laziar, Santa Cruz Province, southern Patagonia. Records from the Late Miocene Puerto Madryn Formation by del Río (1994, 2000) and del Río & Martínez Chiappara (1998b) are referable to a different taxon.

The new species described herein – together with the new species of *Erodona* – comes from rocks referable to the Paraná Formation, exposed along the left margin of the Paraná River between the cities of Paraná and Diamante. These rocks have been variously interpreted as marine (del Río, 1991; del Río & Martínez Chiappara, 1998b), brackish (Aceñolaza, 1976), or as a calcareous reef (Aceñolaza & Aceñolaza, 2000). Our material occurs together with *Portlandia*, *Anadara*, *Amusium*, *Aequipecten*, *Placunanomia*, *Crassostrea*, *Mactra*, and *Chionopsis*. This faunal assemblage is a parautochtonous assemblage generated by tempest flows (Fürsich & Oschmann, 1986; Fürsich, 1993; Simões et al., 1994, 1995). Furthermore, some of the material is clearly reworked as suggested by its taphonomic attributes (e.g., fragmentation of *Crassostrea patagonica*). Thus, the genesis of the bearing sediments may be attributed to a chaotic storm deposit.

The geographic distribution of the species currently referred to *Polymesoda* includes estuaries, lagoons and river mouths at low latitudes along the Atlantic coast of North America, around the Caribbean Sea, the Pacific coast of America between the Gulf of California and northern Peru, and the Indo-Pacific Region (Woodward, 1964; Morton, 1976, 1985; Duobinis-Gray & Hackney, 1982; Ruiz Campos et al., 1998; Rueda & Urban, 1998; Sarcos & Botero, 2005). The occurrence of this genus in locations so far south from its present range, suggests that during the Late Miocene there could have been some pathway connecting these widely separated areas. As all extant species of this genus appear to be restricted to marginal brackish environments or freshwater, migration along the Atlantic coast of South America seems at least unlikely, even more so a Pacific migration around the southernmost tip of the continent. A possible explanation may lie in the probable connection between the Paraná and Amazon basins along a very shallow marginally marine sea arm running along the eastern foothills of the Andes at that time. Such a connection is not accepted by Wesselingh & Salo (2006), but according to Hernández et al. (2005), there may have been an incomplete connection only viable for euryhaline species that tolerated very low salinities or even freshwater conditions, and adapted to shallow estuarine environments. These would have had the dispersal capabilities necessary to invade the Paraná Sea and spread along its coastal areas as far South as the locality studied.

The exposure from where the material was collected carries a rich assemblage of marine taxa that has been assigned a Late Miocene age. These rocks were deposited during the Entrerriense ingression that covered part of central-northern Argentina, reaching as far north as Bolivia, Paraguay and southern Brazil. This taxon appears associated to other taxa that suggest normal salinity, contrasting with the brackish environment preferred by *Polymesoda* and other members of this fauna (i.e., *Erodona*). An alternative explanation to a storm deposit for this faunal assemblage of marine and strictly brackish taxa may be found in fluctuations in sea level. However, the taphonomic attributes of the shells present in the bearing bed – and also its sedimentologic features – point towards the first alternative.

If the marginal brackish conditions reached northwards at least temporarily to the southern boundary of the Amazonian Sea in the Late Miocene, then the paleobiogeographic distribution of this genus can be easily explained, in view of the paleoecological restrictions that such a connection may have imposed on other members of the fauna, while favoring the dispersion of brackish and freshwater taxa.

The extant *Erodona mactroides* lives presently in mainly brackish environments, such as the estuary of the River Plate, where it can be found as far inland as Tigre in Argentina and to the mouth of the estuary at Bahía Samborombón, Argentina, and Montevideo, Uruguay. The other records of this species also include similar environments, such as the Lagoa dos Patos in southern Brazil. According to Sprechman (1978), the range of salinity tolerated by *Erodona mactroides* lies between 3–18 g/l, i.e., typically mixohaline (Da Costa, 1971). However, along the coasts of the Río de la Plata, this range seems to be broader. The paleoenvironment in which the shell bed at La Juanita was deposited appears to be normal marine or nearly so, judging from its fossil assemblage (Table 1). Some of the species may be euryhaline, as is the case of *Polymesoda* (Pérez et al., 2008). However, other taxa included in this same shell bed appear to be clearly marine. This is the case of pectinids and barnacles. Among the shark teeth present are taxa now living in tropical marine environments of northern Brazil. There are chances that the fauna may be a post-mortem mixture of marine and brackish elements due to storm action. However, the shells show no evidence of significant transport, as many of the mollusks – even in cases in which the ligament is relatively weak – were preserved with both valves conjoined. Admittedly, some of the material – as is the case of the specimens of *Crassostrea patagonica* – may have been redeposited, but this is not the case with the great majority of the other taxa.

The presence of *Erodona* in Miocene beds of the Paraná Formation opens questions concerning the origin and evolution of the marine fauna contained in this stratigraphic unit. The present geographic range of the genus seems to be only a small fraction of the original range it occupied in this area of South America. The northernmost fossil record is the whole specimen from La Paz, Entre Ríos (Fig. 1, LP) found in the MLP collection (MLP 7908) within the matrix surrounding a specimen of *Anadara bonplandiana*. Second northernmost record is the specimen (MLP 1819) from Arroyo "Los

Galpones", just south of the city of Paraná (Fig. 1, LG).

In view of the present and fossil distribution of *Polymesoda* and *Erodona*, the presence of these two new species described from rocks included in the Late Miocene Paraná Formation suggests that at the time this unit was deposited there probably was at least a marginal connection between a southern arm of the Amazonian Sea and the northernmost reaches of the Paraná Basin, in a manner similar to the proposed lowland connection between the western Amazonia and the Magdalena basins in northern South America (Wesselingh & Macsotay, 2006), which were possibly linked by a wetland-lake area. The exact nature of the Paraná-Amazon connection remains yet to be ascertained, but the migration of representatives of these genera along the Atlantic coast of South America seems highly unlikely when their peculiar ecological requirements are considered.

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LAND SNAIL FAUNAS IN POLISH FORESTS: PATTERNS OF RICHNESS AND COMPOSITION IN A POST-GLACIAL LANDSCAPE

Robert A. D. Cameron[1]*, Beata M. Pokryszko[2] & Michal Horsák[3]

ABSTRACT

We examine variation in species richness, species composition and distance decay in similarity in forest snail faunas from Poland and a small part of Transcarpathian Ukraine, and their connection with geographical position in relation to Pleistocene refugia. Forest faunas were sampled from sites of standard size in each of ten regions. Data were analysed using DCA and partial CCA, extracting the geographical, climatic and ecological correlates with the major axes. Relationships among site and regional faunas were further examined through the Simpson Index of Similarity. Site species richness shows no significant variation with geographical position or climate, but relates to soil and vegetation characteristics. Composition varies with location; southern highland faunas differ from one another far more than do northern lowland faunas, showing a clear east-to-west pattern of change. The aggregate highland fauna is richer than that of the lowlands, which is a subset of the former. Two intermediate upland regions show different associations, one with the highlands the other with the lowlands. Lowland faunas, even over large distances, are very similar, and all relate more closely to the western end of the highlands than to the east. Disaggregating the fauna into large and small species shows that the former show a stronger geographical pattern than the latter; most universally distributed species are small. Latitudinal variation in regional richness, and longitudinal differentiation among highland faunas relate to distance from glacial refugia. This is not reflected in site species richness, raising questions about the assembly rules for local faunas. The western bias in the relationships of lowland faunas to those of the highlands, the differences between large and small species and the varying rates of faunal turnover within the area studied suggest that patterns of post-glacial dispersal are complex and incompletely understood.

Key words: land snails, Poland, forests, Pleistocene refugia, species richness and composition.

INTRODUCTION

The fauna and flora of northern Europe, arctic-alpine relicts excepted, are mostly products of dispersal from more southerly refuges in the late Pleistocene/early Holocene. While refugia in the Iberian Peninsula, Italy and the Balkans were sources of many immigrants, the status of more northerly areas in Central Europe as refugia is now well attested (Willis et al., 2000; Stewart & Lister, 2001; Willis & Van Andel, 2004). Furthermore, patterns of tree dispersal suggest that the post-Pleistocene process of colonisation is not complete, and has not taken place at equal rates in all directions (Svenning & Skov, 2007; Svenning et al., 2008). Population genetics evidence suggests that range expansion can come about by accidental long-range transport of propagules, a leptokurtic dispersal that can create discontinuous ranges despite continuous environmental suitability (Ibrahim et al., 1996; Hewitt & Ibrahim, 2001).

Hausdorf & Hennig (2003) showed that the land mollusc fauna of northern Europe has nested patterns that can be related to Alpine and Carpathian refugia. Lowland faunas to the north are subsets of the richer regional faunas to the south (Cameron, 2004). While there are a few forest-dwelling species that existed north of the mountain ranges through the last glaciation (Moine et al., 2008) and rather more that survived in the Hungarian Carpathian Basin, not far from the area north of the Carpathians in which our samples were made (Willis et al.,

[1]Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, U.K. & Department of Zoology, The Natural History Museum, London SW7 5BD, U.K.
[2]Museum of Natural History, Wrocław University, Sienkiewicza 21, 50-335 Wrocław, Poland
[3]Department of Botany and Zoology, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic
*Corresponding author: r.cameron@sheffield.ac.uk

1995, 2000; Pokorný, 2002; Sümegi & Krolopp, 2002), most present-day forest faunas exist in places unoccupied by those species until approximately 10,000 BP or later. Although the western, alpine or eastern affinities of some species can be identified (Hausdorf & Hennig, 2003), there are many for which the origins are obscure, and northern faunas have accumulated from diverse sources (Waldén, 1986). There are many species with discontinuous ranges, not easily explained in terms of the present environment (Wiktor, 2004).

In this paper, we use data from forest sites in Poland and part of Transcarpathian Ukraine to examine the roles of dispersal and ecology in determining patterns of species richness, composition and faunal turnover in an area that covers a wide range of distances from possible sources.

MATERIAL AND METHODS

Samples of land snails were taken in 161 forest sites (149 in Poland, 12 in Transcarpathian Ukraine) between 2001 and 2007. Sites were located in clusters by geographical region, though these regions varied considerably in size. Figure 1 shows the locations of the regions (details of the locations of sites are given in Appendix 1). The Sudetes, Pieniny, southeastern Poland and Transcarpathian Ukraine regions are mountainous, and are regarded as highland regions. Kaszuby, Romnicka, Białowieża and Roztocze, in the Northern European Plain, are regarded as lowland. Ojców, at the southern end of the Kraków – Częstochowa upland, and Holy Cross (the isolated Góry Świętokrzyskie, reaching a maximum 695 m) are regarded as upland. Analyses of material from Białowieża and Kaszuby have been published elsewhere (Cameron & Pokryszko, 2004, 2006), but the data are included here.

Each site was selected to yield a fauna typical of its region, thus avoiding obvious gross disturbance or monoculture plantations. Each site, of approximately 400 m^2, was searched by two people for between one and two hours, and between 10 and 20 litres of litter were collected from the site and sieved. This variation in sampling effort was designed to ensure adequate numbers for inventory at each site (Cameron & Pokryszko, 2005). Methods of processing samples otherwise follow Cameron & Pokryszko (2004). Slugs are not included in our analyses; the sampling methods used do not give reliable results for inventories. Nomenclature generally follows Wiktor (2004); exceptions are noted in Appendix 2.

Location and altitude were determined from maps, and climatic data from Hijmans et al. (2005). Apart from these continuous variables, ordinal scores were recorded for slope, amount of exposed rock or scree, of limestone substrate, of disturbance and of permanently waterlogged ground. Ordinal scores were also recorded for presence/dominance of tree species and field layer plants typical of particular soil conditions. Combinations of these scores were also used. The presence or absence of a stream or river, of large rotting timber, and of predominantly bare ground under the canopy were also recorded. In total, 35 geographical, climatic and environmental variables were used (Appendix 1, which also shows a PCA analysis of relationships between them).

The completeness of inventories was examined using the Chao 1 Estimator (Colwell & Coddington, 1994; Walther & Moore, 2005), using the number of species in each sample represented by one and by two individuals. The number of such species in each sample was very small (often zero), and the errors associated with the estimate in this situation are very large. Following Cameron & Pokryszko (2005) and Cameron et al. (2006), we have therefore taken the mean numbers of each per site by region to give an approximate indication of the number of species missing (data not shown). These estimates are very low, and show no relationship to the median sample size in each region. We have made no corrections to allow for missing species.

Most species in our array showed a unimodal response in ordination (showing a single mode in the mid range, rather than a linear response). We therefore used ordination techniques (Correspondence Analysis, Ter Braak & Prentice, 1988) based on such a response model (Lepš & Šmilauer, 2003). The species-by-sites matrix was analysed by Detrended Correspondence Analysis, DCA, (numbers transformed as log + 1; rare species downweighted) to expose the major compositional gradients in the data. We chose an indirect gradient analysis (i.e. DCA) since our study is not hypothesis-testing research and no guarantee can be given that all relevant factors associated with each major compositional gradient have been measured (for details, see Økland, 1996). To explain the gradients in the species data, the correlations with geographical, climate, and environmental variables were examined using Spearman correlations (r_S) with site scores on the first four DCA axes. The matrix of two-dimensional geographical coordinates was completed by adding all terms for a cubic trend surface regression and these nine parameters were used as descriptors of spatial structure (for details, see Borcard et al., 1992). To separate the independent variation caused by particular external factors (i.e., environment, climate, and space) we used the variation partitioning approach using separate Canonical Correspondence Analyses (partial CCAs) (Borcard et al., 1992). Details of the CCAs are given in Appendix 3 (Table 4). The CANOCO 4.5 package (Ter Braak & Šmilauer, 2002) was used for ordination techniques and STATISTICA 7 (Hill & Lewicki, 2007) for unidimensional analyses. Sequential Bonferroni corrections of the significance level were used for multiple comparisons of environmental variables (Holm, 1979).

Further analyses use Whittaker's Index of Diversity for among site diversities within regions and the Simpson indices of similarity between regional faunas (Index 22 in Koleff et al., 2003: 370), using both the total inventory for each and mean site-by-site indices within each region. The Simpson Index counteracts the effects of different richness among sites, and enables variance estimates to be made. A Mantel test was used to detect spatial autocorrelation and distance decay, the rate of change in faunal composition with distance (Nekola & White, 1999).

RESULTS

The 161 sites produced a total of 86,191 individuals of 111 species (Appendix 2). Of these 111 species, 12 were found in only one site, and 10 were represented in the whole array by fewer than 10 individuals. While some of these can be regarded as rare forest species, others are typical of open and disturbed habitats. Nine species in this category (indicated in Appendix 2) have been eliminated from the array before analysis, which uses the remaining 102.

Species Richness

Table 1 shows the mean, median and the range of site species richness by region. There is considerable variation both among and within regions, but there are no significant relationships with either latitude or longitude, or with altitude. Only the environmental factors shown in Table 2 have significant effects. Those showing positive associations are themselves correlated. Table 2 also shows their combined effect, where only the combination of high scores over all factors produces a significant effect. The contrast between the two upland regions (Ojców and Holy Cross: Table 1) has an ecological cause; samples from Ojców are all from mixed forests on rocky limestone, while most of those from Holy Cross come from relatively small patches of partly deciduous forest in an area of acidic soils dominated by conifers. The only negative

TABLE 1. Basic data for the samples used in this study.

Region	No. of Samples	Mean Nos.	Median Nos.	Range Nos.	No. of Species	Mean per Sample	SD	Median	Range
Lowland									
Kaszuby	14	529	494	247–894	43	24.7	4.2	26	17–31
Romincka	12	830	766	474–1702	50	31.1	3.5	32.5	24–35
Białowieża	17	272	236	126–669	46	24.5	4.8	25	13–32
Roztocze	13	612	489	253–2257	52	22.9	3.5	22	18–29
Upland									
Holy Cross	6	357	371	178–543	38	19.0	3.7	18.5	15–25
Ojców	7	982	932	804–1247	44	36.4	2.0	37	33–39
Highland									
Sudetes	33	390	334	141–953	73	25.9	5.2	24	17–38
Pieniny	15	318	309	171–561	60	31.3	5.0	30	26–43
S.E. Poland	32	521	477	229–1018	71	30.8	4.8	31	21–43
Ukraine	12	307	284	77–774	44	25.8	4.2	26	16–31

TABLE 2. Mean site species richness in relation to environmental variables. In each case, 0, absent; 1, present but not abundant; 2, dominant or abundant. MDW: mixed deciduous woodland containing *Tilia, Fraxinus* or *Ulmus* and without a single dominant species. Rich: the occurrence of herbaceous plants indicating nutrient rich soil. Lime: limestone substrate at surface. Comb: the added scores for rocks, limestone, MDW and rich (maximum 8). Dist: 1 indicates evidence of recent disturbance. The overall mean for all sites is also given. *, **, ***, significant differences from the other caregories at $P < 0.05, 0.01, 0.001$ respectively.

	n	Mean	SD
Rocks			
2	31	30.9**	5.96
1	37	26.2	5.77
0	93	26.7	5.43
MDW			
2	67	28.6*	6.62
1	72	27.0	4.89
0	22	25.1	5.58
Comb			
7-8	13	35.6***	3.95
5-6	22	28.2	5.32
3-4	61	26.7	5.61
0-2	65	26.1	5.27
Lime			
2	27	31.0**	6.11
1	12	24.3	4.55
0	122	26.9	5.58
Rich			
2	32	32.1***	5.36
1	73	26.2	5.02
0	56	26.3	5.86
Dist			
1	39	25.7*	5.84
0	122	27.9	5.87
All	161	27.4	5.84

factor to achieve significance relates to disturbance, which was never extreme.

Composition: Site-by-Site Analyses

Figure 2 shows the distribution of sites on the first two axes of the DCA analysis, coded by region. It is evident that much of the variation in the data is among regions. The first axis separates the highland regions along an east-west axis, but with all lowland regions at the left end regardless of location; the second axis reflects a complex interaction of latitude, longitude and environment. There is a small overlap between the main lowland cluster and that of the Sudetes. Of the two upland areas, Ojców is in the highland sequence, and occupies an appropriate geographical position between the Sudetes and the Pieniny (Fig. 1). Holy Cross sites generally fall into the lowland cluster. Figure 2 also shows the relationships of the most significant environmental variables to the DCA axes (correlations among all explanatory variables and sites scores on the first four ordination axes are available in Appendix 3 (Table 1). DCA analysis based on presence and absence, CCA analysis, and PCoA analysis of presence and absence data, using site-by-site values of the Simpson Index of similarity (data not shown) produce very similar patterns; presence or absence of species accounts for most of the pattern observed. The clear regional grouping of sites implies a degree of spatial autocorrelation, or, reversing the emphasis, of distance decay in similarity, and an overall Mantel test on Simpson similarities in the whole array gives $r = 0.576$ ($P < 0.001$).

The independent effects of different categories of external variables were determined and tested by partial CCAs (details: Appendix 3, Table 4). The pure effect of location, independent of other variables, has a major influence on distribution of species. Hence, further analyses examine the geographical pattern in more detail.

Composition: Regional Analysis

There are clear differences in the amount of heterogeneity within regions. Table 3 demonstrates this, comparing values of Whittaker's Index, its variant $I_{max,}$ and of the mean site-by-site Simpson Index for each region. The size of the region over which samples were taken is important, as is the range of forest types sampled in each. However, there is no clear geographical pattern within regions (data not shown), and in all regions the richest site contains more than half of all species we found in its region; we therefore use total regional faunas to explore patterns in more detail.

At the coarsest scale, there are clear differences between lowland and highland regions. Table 4 shows that while most species in the lowlands occur in at least three of the four

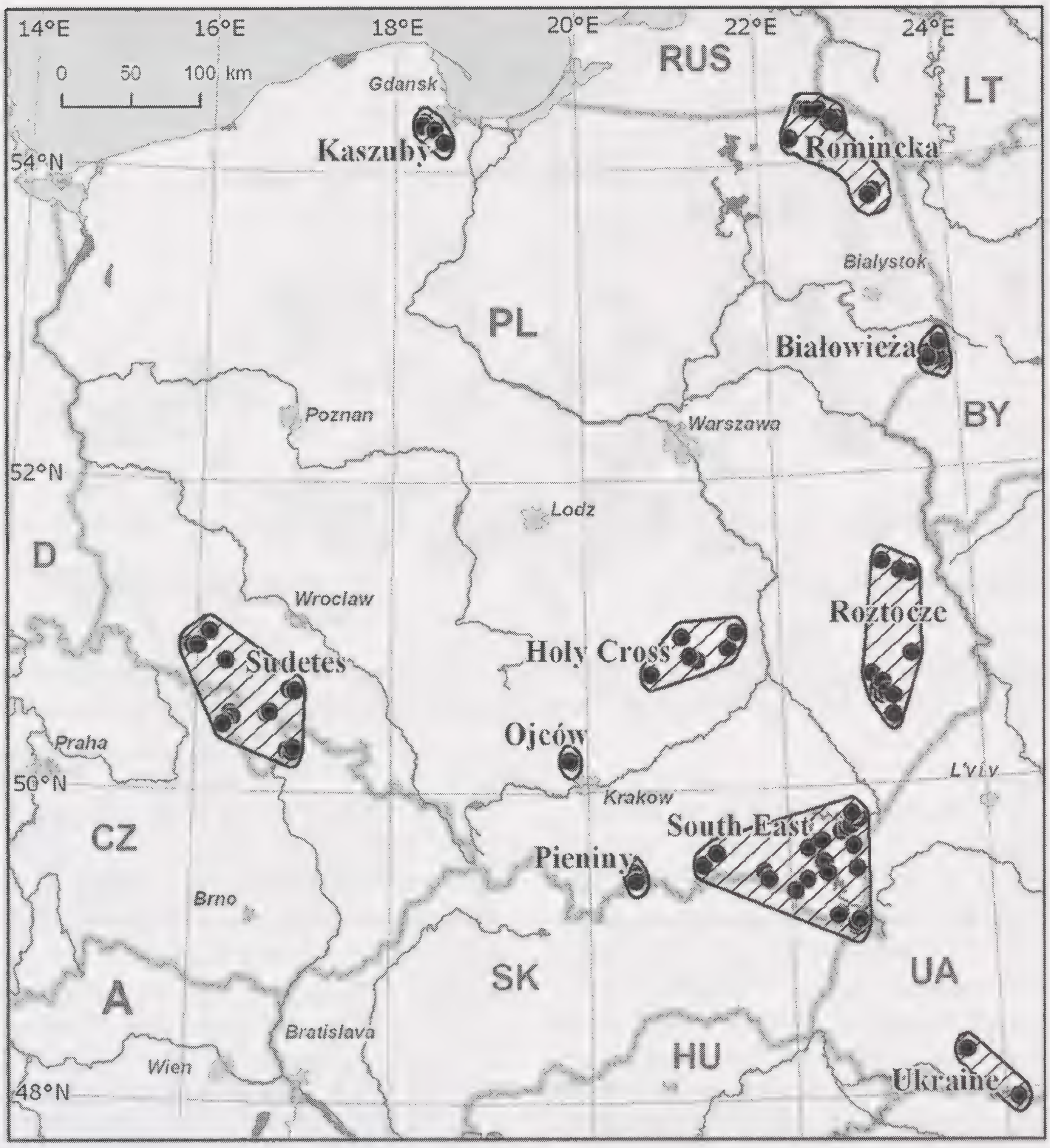

FIG. 1. Map showing the location and approximate size of regions within which samples were taken.

TABLE 3. Values of Whittaker's index, I_{max}, and mean site-by-site Simpson indices for each region. SE, Standard Error of the mean Simpson Index.

Region	No.	Whitt.	Whitt. max	Mean Simpson	SE
Kaszuby	14	1.74	1.39	0.81	0.010
Romincka	12	1.61	1.43	0.81	0.010
Białowieża	17	1.88	1.44	0.84	0.007
Roztocze	13	2.27	1.79	0.68	0.012
Holy Cross	6	2.00	1.52	0.71	0.031
Ojców	7	1.20	1.13	0.91	0.009
Sudetes	33	2.82	1.92	0.69	0.006
Pieniny	15	1.92	1.40	0.79	0.009
SE Poland	32	2.31	1.65	0.72	0.004
Ukraine	12	1.71	1.42	0.79	0.011

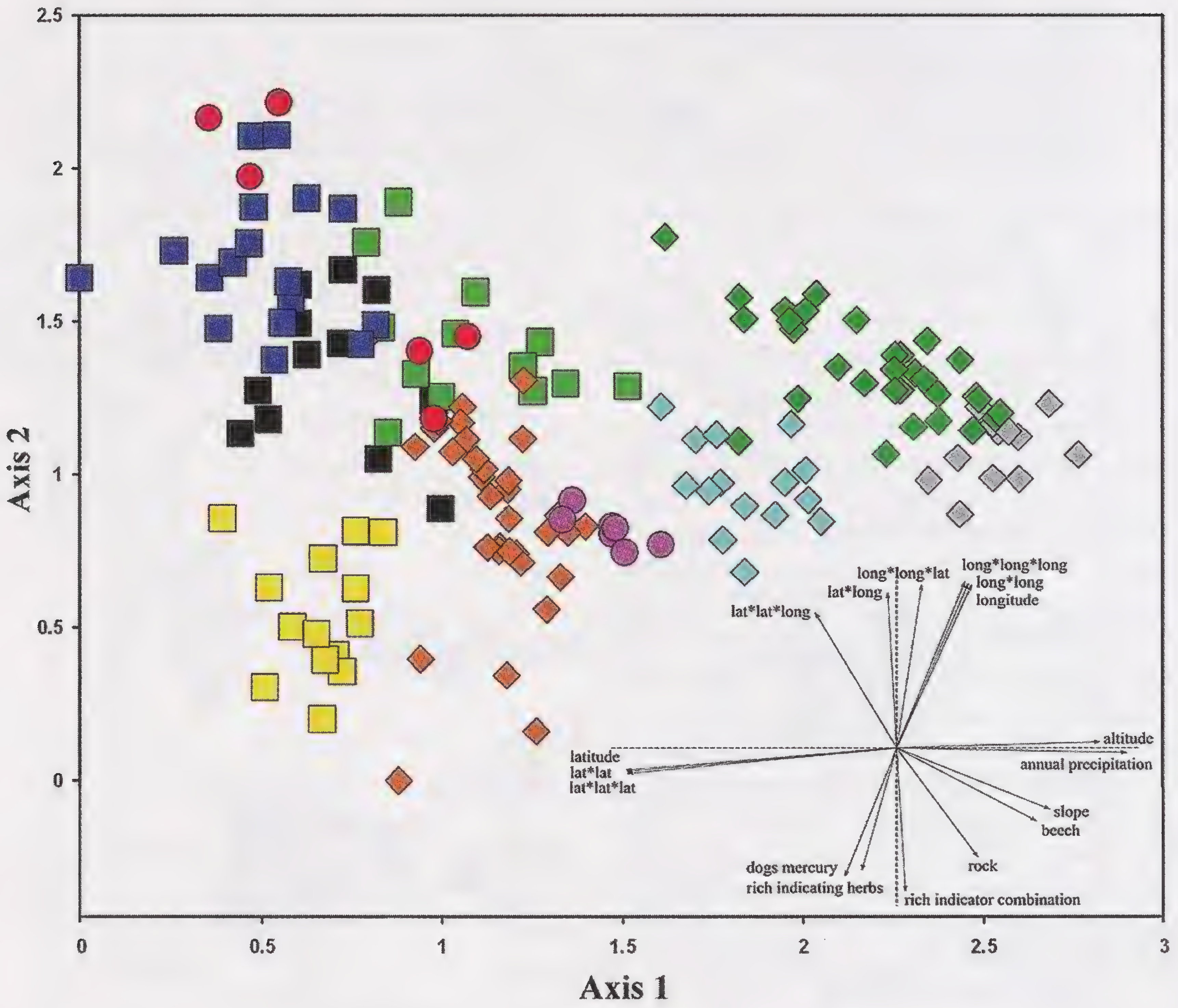

FIG. 2. Plots of site scores on the first two axes of a DCA, using log-transformed abundances. Explanatory variables significantly and highly correlated ($r_S > 0.5$) with the first two DCA axes are shown in the bottom right. Sites are coded by region. Diamonds, highland regions; circles, upland; squares, lowland. Black, Romincka; dark blue, Białowieża; light green, Roztocze; yellow, Kaszuby; red, Holy Cross; pink, Ojców; orange, Sudetes; pale blue, Pieniny; dark green, S.E. Poland; grey, Ukraine.

TABLE 4. Patterns of distribution of species among lowland, highland and upland regions. A: Species unique to each category. Unique in the lowland and highland columns takes no account of presence in either upland region. B: Numbers of species occupying 1, 2, 3, 4 regions within the category concerned. Highland widespread indicates species also present in one or more lowland regions.

A	Lowland	Highland	Highland ex Ukraine	Ojców	Holy Cross
No. of samples	56	92	80	7	6
Total species	64	97	93	44	38
Unique	3	36	32	1	1
% Unique	4.7	37.1	34.4	2.3	2.6
Not in Lowland				10 (23%)	3 (8%)

B	All Lowland	Highland widespread	Highland only	All Highland
1 Region	10	8	16	24
2 Regions	11	12	9	21
3 Regions	13	18	8	26
4 Regions	30	23	3	26

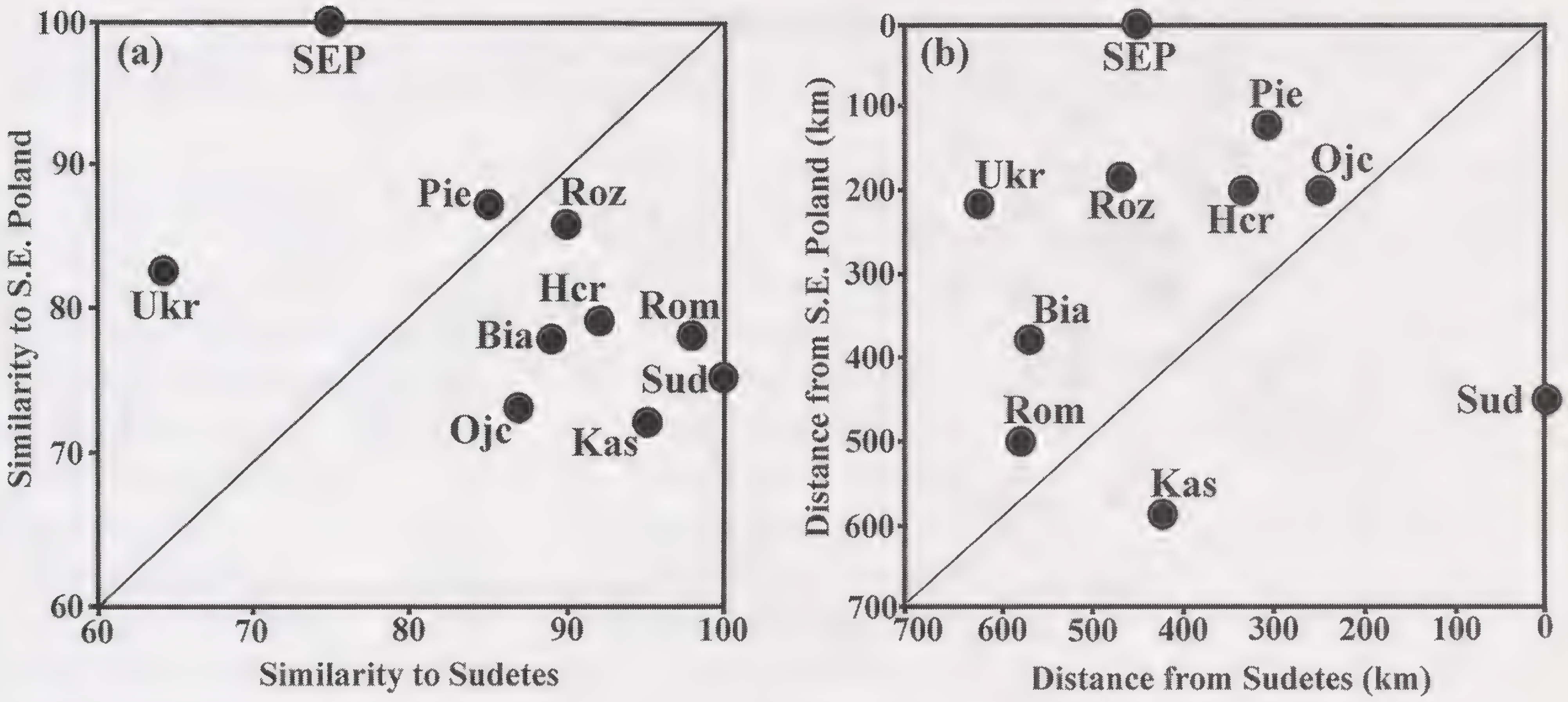

FIG. 3. The relationships of regional faunas to those of the Sudetes and of S.E. Poland, (a), Simpson Index; (b), mean geographic distance; the diagonal line indicates equal distance/similarity.

lowland regions, the split is more even in the highlands. Within the highlands, species also found in the lowlands are more widespread among the former. Only three species found in the lowlands are missing from the highlands; one of these is the very rare wetland specialist *Cochlicopa nitens.* The other two, *Aegopinella nitidula* and *Clausilia bidentata,* are species with western affinities (Wiktor, 2004). In contrast, 36 species are unique to the highlands, and only four of these are restricted to Ukraine; the lowland fauna is a subset of the highland fauna. The upland regions, Ojców and Holy Cross, are intermediate, though differing from one another greatly; Ojców has a much higher proportion of species not found in the lowland regions.

At a finer grain, there are differences among regions. Table 5 shows among-region similarities using Simpson Indices for the whole fauna of each region. The four lowland regions show strong affinities with each other. They also show strong affinities to the Sudetes, and lower affinities to the other highland regions (Fig. 3). Kaszuby and Romincka have faunas that are little more than subsets of that of the Sudetes, although the latter is the furthest away from the Sudetes. Similarly, both upland regions relate more to the Sudetes than to other highland regions; this accords with geographical distance for Ojców, but not for Holy Cross. Within the highland regions, in contrast, similarity is clearly related to geographical distance.

TABLE 5. Simpson indices between total regional faunas. In general, differences between indices greater than 0.025 are significant. The top third of values are in bold.

A	rom	bia	roz	hcr	ojc	sud	pie	sep	ukr
kas	**0.91**	**0.93**	0.79	0.74	0.56	**0.95**	0.72	0.74	0.47
rom		**0.89**	**0.84**	**0.84**	0.64	**0.98**	0.78	0.80	0.55
bia			**0.87**	0.76	0.57	**0.89**	0.78	0.80	0.50
roz				0.82	0.59	**0.90**	0.77	**0.87**	0.61
hcr					0.55	**0.92**	0.76	0.79	0.45
ojc						**0.89**	**0.84**	0.77	0.52
sud							**0.85**	0.76	0.64
pie								**0.88**	0.75
sep									0.82

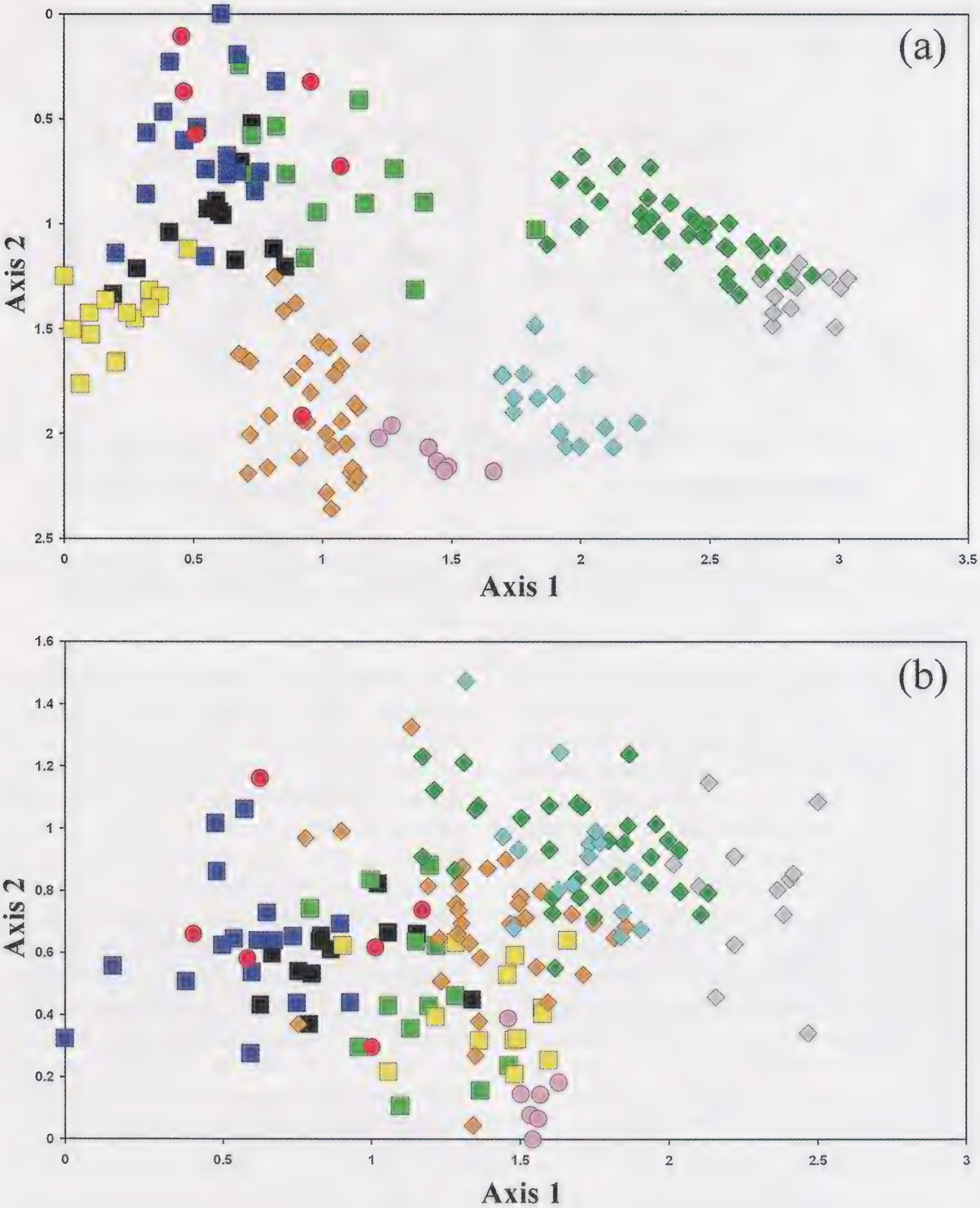

FIG. 4. Plots of site scores on the first two axes of a DCA using (a) large species and semislugs only, and (b) using small species only (see text). Symbols as in Fig. 2.

Although there is spatial autocorrelation overall (see above), these patterns indicate that the rate of distance decay in similarity varies greatly from any one region to others. The Simpson index between regional faunas shows only a weak association with distance ($r_S = -0.244$, $P > 0.1$) and this is largely an effect of Ukraine; without this region there is no connection with distance ($r_S = -0.04$, $P > 0.8$).

Composition: Species Analysis

Among the 102 species included in the analysis, 12 were recorded in all 10 regions while 16 were found only in one (Table 6). Examples of both widely and narrowly distributed species can be found in all those family or superfamily groupings with numerous species. Positions on the DCA biplot (not shown) reflect geographical

TABLE 6. Universal and unique species (by region). The first 10 universal species are also the most frequent by sites occupied. Asterisked species are small (see text). There were no species unique to Pieniny, Kaszuby, Romincka or Roztocze. For comment on *Vestia* sp. see Appendix 2.

Universal Species	Site Freq.	Regs	Unique Species	ukr	sep	sud	bia	ojc	hcr
**Punctum pygmaeum*	159	10	*Vestia* sp.	X					
**Carychium tridentatum*	152	10	*Mastus bielzi*	X					
**Aegopinella pura*	148	10	*Drobacia banatica*	X					
**Columella edentula*	133	10	*Acicula perpusilla*	X					
**Acanthinula aculeata*	129	10	*Carpathica calophana*		X				
**Vertigo pusilla*	126	10	*Trichia bakowskii*		X				
**Euconulus fulvus*	125	10	*Pagodulina pagodula*		X				
Cochlodina laminata	123	10	*Eucobresia diaphana*			X			
Vitrina pellucida	120	10	*Causa holosericum*			X			
Cochlicopa lubrica	100	10	*Helicodonta obvoluta*			X			
**Vitrea crystallina*	70	10	*Oxychilus draparnaudi*			X			
Helix pomatia	75	10	*Cochlodina costata*			X			
			Cochlicopa nitens				X		
			Pyramidula pusilla					X	
			Vestia elata						X

distribution. The only association with extent of distribution that we can detect is size. For purposes of analysis, we have followed Hausdorf & Hennig (2003) in taking the 26 species with maximum shell dimension less than 5 mm to be small, and regarding all others, including all semislugs, as large. Seven of the ten most frequent species by sites are small, as are eight out of the 12 found in all ten regions (Table 6). These proportions are significantly different from those in the remainder (χ^2 test, $P < 0.001$ in each case).

When sites are analysed by DCA using small and large species separately (Fig. 4, Appendix 3), the effects of size are manifest; for large species alone, sites in lowland regions cluster closely together, while the distinctions between them and highland regions, and among the highland regions are more marked than for the fauna as a whole (Fig. 2). In contrast, the pattern for small species is much less clear, with more overlap among regions. Only latitude, slope and precipitation show major relationships to the axes in the latter, and that for latitude is much weaker than for large species ($r_S = 0.69$, as compared with 0.90). Simpson Indices for small species alone are higher than those for the fauna as a whole; for some lowland comparisons, species compositions are identical (data not shown).

DISCUSSION

Five major findings emerge from our analyses:

(1) The species richness of individual sites varies rather little, and relates to ecological conditions rather than geographical position.

(2) Composition, however, relates to geography, in large part independently of present climate or other ecological constraints.

(3) Highland regions are richer than lowland regions; the former are much closer to putative Pleistocene refugia.

(4) Distance decay in similarity varies; lowland faunas are all very similar, and show a greater similarity to that of the Sudetes than to those of other highland regions further east, even when the latter are nearer. Highland regions are more differentiated.

(5) Large species show more differentiation among regions than small species.

For site species richness, our results strengthen those of Pokryszko & Cameron (2005), who used less standardised data over a larger area. At the scale of small sites, forest snail faunas show no pronounced change in richness with latitude. Site faunas from England or southern Sweden are as rich as those 600 km or more to the south (Waldén, 1981; Cameron et al., 2006). While comparable data are sparse, the relative stability of species richness in forest sites across Europe stands in sharp contrast to the strong latitudinal trend for overall regional species richness within the continent (Cameron, 2004). Only in the far north (above 60°), where forest types are very restricted, or in the far east, in the southern Urals, very far from any refugia and with a more extreme climate (Horsák et al., in press), is local richness significantly lower. Distance from putative refugia has little effect on local richness within the limits of our study area. This contrast in pattern between regional and local richness might indicate that local, biotic interactions are limiting local richness independently of the regional pool (Srivastava, 1999). However, Hillebrand (2005) has warned against any simplistic interpretation of such relationships, and since snail faunas often include many restricted endemics and snails have poor powers of active dispersal, the concept of a regional fauna in this context is hard to define: what is actually available at site x? Evidence for interspecific competition in land snails is sparse and mainly confined to examples of closely related or similar-sized species, usually in rather stressed environments with limited faunas (Solem, 1984; Baur & Baur, 1990; Cameron et al., 2003). The classic scepticism of Boycott (1934) about the role of competition in structuring snail faunas has not been refuted. Nevertheless, our study emphasises the relative invariance of site species richness in forest snail faunas across many climatic and biogeographic regions (Stanisic et al., 2007), in contrast to many other organisms (Ricklefs, 2004).

In contrast, regional richness and composition are clearly related to geographical position. There is a coherent pattern of differentiation along the Sudetes-Carpathian chain. Inevitably, more unique species are found at either end of this transect than at the centre; the Sudetes support species with Alpine affinities, whereas Ukraine has species of Balkan affinities. Ukrainian faunas are the most distinct, and come from the only region in our study to lie south of the mountain chain crest. Given the strong showing of purely geographical factors in the partition of variance, this pattern appears to be a product of distances from diverse refugia to the south, east and west. Hausdorf & Hennig (2003) detected foci of nestedness in the eastern Alps and in the Carpathians. There are, however, numerous forest species that are spread across the ranges (and into the lowlands). The evidence that forests persisted throughout the last glaciation in areas just south of our study area has become overwhelming (Willis et al., 2000; Willis & Van Andel, 2004; Birks & Willis, 2008). Both palaeobotanical and palaeomalacological data show that even at the glacial maximum there was a zone of forests consisting of larch, pine, and spruce in the Carpathians (Jankovská & Pokorný, 2008; Kuneš et al., 2008). Ložek (2006) confirmed that between the loess steppe belt at the foot of the south side of the Carpathians and the zone of alpine grasslands a zone of montane to subalpine woodlands at 600–1,600 m a.s.l. persisted during the glacial period. He found in the sediments of this zone a mixture of glacial open-country elements and several woodland species that are considered characteristic of warm interglacial periods. Sümegi & Krolopp (2002) similarly reported the occurrence in the Carpathian basin of species now found in some of our sites. In Bohemia, however, the region immediately south of the Sudetes, there is no evidence of the survival of such forests or the snail faunas that accompanied them (Ložek, 2006). Although the many studies within our area reveal only open-country faunas north of the Carpathian watershed before approximately 11,000 BP (Alexandrowicz, 2004), it is evident that dispersal into developing Holocene forests could involve shorter distances, over a wider front, than previously thought.

In contrast, lowland faunas are less differentiated, and contain a higher proportion of widespread species, many ranging across the whole Northern European Plain (Pokryszko & Cameron, 2005; Horsák et al., in press). That they form subsets of those in the highlands conforms to the findings of Hausdorf & Hennig (2003). The novel feature here, however, is the stronger relationship to the fauna of the Sudetes than to those of regions further east, to the extent that measures of differentiation among faunas show little relationship to distance. Many species found in the Carpathians have failed to move north, despite apparently suitable habitats. Mountains in the Sudetes, and in the neighbouring western Beskids, are generally lower than those further east, perhaps offering fewer barriers to the dispersal of (generally low altitude) forest species. What is clear, however, is that Holocene dispersal across the Polish lowlands has not been uniform in speed or direction. A western bias in source of colonisation has also been reported for early-colonising trees (Kullman, 2002). Within the highlands, species with limited ranges contribute to a rapid rate of turnover. Hausdorf & Hennig (2004) found little evidence for vicariance in the Northern European snail fauna, but evidence from elsewhere (Weir, 2006) suggests that highland faunas may differentiate by isolation caused by climatic change more readily than those in lowlands.

These findings, coupled with the evidence that small species have dispersed more rapidly than large ones, raise questions about the role and mechanics of dispersal in shaping the present faunas. This wider distribution of small species can be further linked with their ability to survive under cold conditions. We see no differences between the small number of large species that have spread over most of the area, and those that remain confined to highlands close to earlier refuges. Within the lowland and upland faunas, there are cases of disjunct distributions, sometimes involving gaps of 200 km or more (Wiktor, 2004). Thus *Chilostoma faustinum,* found along the mountain chain, is found in the lowlands in Roztocze, which is nearby, and also in Rominсka (Marzec, 2005), which is the most distant region, but not in Białowieża, which lies in between. *Vestia elata* and *Balea fallax,* both generally restricted Carpathian species, are known from single sites in the Holy Cross and Roztocze respectively. *Ena montana,* not found in any of our lowland regions, is recorded even further north in Latvia and Lithuania (Skujienė, 2002).

We can identify possible mechanisms to account for some of these patterns. The central belt of Poland is more fertile than the far north, and far more intensively cultivated. Thus, disjunct distributions can result from destruction of populations in the middle of ranges (Cameron & Pokryszko, 2006). Humans can act as long-distance dispersal agents; this is well documented for a few species (e.g., Ożgo, 2005), but is probably not a significant feature for forest faunas. Climate was neither stable nor continuously warming through the Holocene (Walker, 1995), and expansions and contractions of ranges in response can leave isolated relicts in favoured locations. In this context, the occurrence of *Semilimax kotulae* and *Vestia elata* in the Holy Cross region, and of many highland species at Ojców is significant. While we cannot dismiss the possibility that these processes account for such discontinuous distributions, there is a further mechanism, the long-distance dispersal of propagules, which could produce similar patterns (Ibrahim et al., 1996; Hewitt & Ibrahim, 2001). Certainly, there are cases in which isolated occurrences of some large species (e.g., *Ena montana* mentioned above) do not relate only to a demonstrable refuge. We cannot assume a stable balance; sites in parts of the previously glaciated or periglacial areas north of the European mountain ranges may be "full", but others may still be open to colonisation, a process now hindered by human intervention (Giesecke et al., 2007; Svenning et al., 2008).

As Nekola & White (1999) demonstrated, analyses of distance decay require consideration of differences in dispersal ability and environmental tolerance. The mechanisms of dispersal and the constraints on successful establishment in land snails remain to be determined.

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APPENDIX 1

Environmental and locality data

TABLE 1. Descriptive statistics for explanatory variables used in the analyses. Wet indicator combination: wet, alder/willow, wet indicating herbs. Rich indicator combination: rock, limestone, mixed deciduous wood, ash, dog's mercury, rich indicating herbs, timber. Poor indicator combination: conifers, acid indicating herbs, bare ground.

	Minimum	Lower quartile	Mean	Median	Upper quartile	Maximum
Environment						
Rock	0.0	0.0	0.6	0.0	1.0	2.0
Limestone	0.0	0.0	0.4	0.0	0.0	2.0
Slope	0.0	0.0	1.0	1.0	2.0	2.0
Wet	0.0	0.0	0.6	0.0	1.0	2.0
Disturbance	0.0	0.0	0.3	0.0	0.0	2.0
Conifers	0.0	0.0	0.5	0.0	1.0	2.0

(continues)

(continued)

	Minimum	Lower quartile	Mean	Median	Upper quartile	Maximum
Beech	0.0	0.0	0.8	1.0	2.0	2.0
Mixed deciduous wood	0.0	1.0	1.3	1.0	2.0	2.0
Alder/willow	0.0	0.0	0.6	0.0	1.0	2.0
Ash	0.0	0.0	0.3	0.0	1.0	2.0
Wet indicating herbs	0.0	0.0	0.4	0.0	1.0	2.0
Dog's mercury	0.0	0.0	0.7	1.0	1.0	2.0
Rich indicating herbs	0.0	0.0	0.9	1.0	1.0	2.0
Acid indicating herbs	0.0	0.0	0.3	0.0	1.0	2.0
Altitude	80.0	175.0	341.6	290.0	470.0	780.0
Wet indicator combination	0.0	0.0	1.5	1.0	2.0	6.0
Rich indicator combination	1.0	3.0	5.4	5.0	7.0	13.0
Poor indicator combination	0.0	1.0	1.7	2.0	2.0	5.0
Climate						
Mean Annual Temp. (°C)	4.3	6.1	6.6	6.7	7.1	8.0
Mean Max. Temp. Warmest Month	17.7	21.3	21.9	22.1	22.8	23.9
Mean Min. Temp. Coldest Month	-9.9	-8.6	-7.9	-8.0	-7.5	-5.4
Annual Precipitation (mm)	515.0	583.0	674.0	659.0	765.0	854.0
Coefficient of variation, seasonal precipitation	30.0	33.0	37.4	37.0	41.0	49.0
Space						
Latitude	47.9	49.5	50.9	50.5	52.7	54.3
Longitude	15.9	18.4	20.8	21.8	23.0	24.1
Nominal Environmental						
Stream	0.0	0.0	0.6	1.0	1.0	1.0
Timber	0.0	0.0	0.3	0.0	1.0	1.0
Bare	0.0	0.0	0.3	0.0	1.0	1.0

→

TABLE 2. PCA of all explanatory variables in the 161 forests sites. In total, 35 geographical, climatic and environmental variables were used and they were subjected to principal components analysis (PCA) of the correlation matrix (centred and standardised) to show the main gradients of their variation and the relationships among individual variables. Eigenvalues of axes (in parentheses), Spearman correlations of continuous and ordinal variables with the first four PCA axes (r_S) and their significances (P) are shown. Significant correlations after Bonferroni correction are in bold (the cut level was P = 0.0016). Mann-Whitney U test was used for testing relationships between nominal variables and sites scores on the PCA axes. Significant relationships (P = 0.05) are in bold.

	PCA1		PCA2		PCA3		PCA4	
	(0.257)		(0.186)		(0.111)		(0.086)	
Continuous and ordinal	r_s	P	r_s	P	r_s	P	r_s	P
Environment								
Rock	**-0.80**	<0.001	-0.18	0.020	-0.18	0.021	-0.10	0.199
Limestone	**-0.53**	<0.001	-0.20	0.011	**-0.25**	<0.001	-0.24	0.002
Slope	**-0.63**	<0.001	**-0.42**	<0.001	-0.10	0.197	-0.05	0.560
Wet	**0.60**	<0.001	0.19	0.018	**0.53**	<0.001	-0.22	0.004
Disturbance	0.03	0.734	-0.07	0.400	**-0.25**	<0.001	-0.14	0.068
Conifers	**0.51**	<0.001	0.13	0.097	0.05	0.506	**0.30**	<0.001
Beech	**-0.44**	<0.001	**-0.56**	<0.001	-0.04	0.591	**0.30**	<0.001
Mixed deciduous wood	-0.12	0.132	**0.54**	<0.001	**-0.27**	<0.001	-0.20	0.011
Alder/willow	**0.49**	<0.001	**0.26**	<0.001	**0.49**	<0.001	**-0.36**	<0.001
Ash	0.15	0.055	0.16	0.042	0.01	0.972	**-0.49**	<0.001
Wet indicating herbs	**0.39**	<0.001	-0.16	0.041	**0.54**	<0.001	**-0.43**	<0.001
Dog's mercury	**-0.47**	<0.001	**0.49**	<0.001	**-0.29**	<0.001	-0.16	0.043
Rich indicating herbs	**-0.34**	<0.001	**0.36**	<0.001	**-0.44**	<0.001	-0.08	0.286
Acid indicating herbs	0.23	0.003	-0.11	0.169	0.06	0.471	**0.42**	<0.001
Altitude	**-0.55**	<0.001	**-0.64**	<0.001	**0.28**	<0.001	-0.01	0.937
Wet indicator combination	**0.57**	<0.001	0.17	0.033	**0.59**	<0.001	**-0.36**	<0.001
Rich indicator combination	**-0.60**	<0.001	**0.38**	<0.001	**-0.42**	<0.001	**-0.25**	<0.001
Poor indicator combination	-0.01	0.943	**-0.47**	<0.001	-0.01	0.887	**0.62**	<0.001
Climate								
Mean Annual Temp. (°C)	-0.07	0.387	0.01	0.908	**-0.50**	<0.001	**-0.50**	<0.001
Mean Max. Temp. Warmest Month	**0.27**	<0.001	-0.20	0.011	**-0.48**	<0.001	**-0.43**	<0.001
Mean Min. Temp. Coldest Month	-0.18	0.024	**0.70**	<0.001	-0.20	0.011	0.00	0.953
Annual Precipitation (mm)	**-0.40**	<0.001	**-0.67**	<0.001	**0.26**	<0.001	0.02	0.791
Coefficient of variation, seasonal precipitation	**-0.63**	<0.001	-0.09	0.278	0.03	0.665	**-0.28**	<0.001
Space								
Latitude	**0.38**	<0.001	**0.81**	<0.001	-0.12	0.124	**0.25**	<0.001
Longitude	**0.71**	<0.001	**-0.48**	<0.001	**-0.34**	<0.001	-0.10	0.227
Lat.*Long.	**0.83**	<0.001	**-0.31**	<0.001	**-0.34**	<0.001	-0.06	0.463
Lat.*Lat.	**0.38**	<0.001	**0.81**	<0.001	-0.12	0.124	**0.25**	<0.001
Long.*Long.	**0.71**	<0.001	**-0.48**	<0.001	**-0.34**	<0.001	-0.10	0.227
Lat.*Lat.*Long.	**0.88**	<0.001	-0.15	0.063	**-0.31**	<0.001	0.01	0.870
Lat.*Long.*Long.	**0.79**	<0.001	**-0.36**	<0.001	**-0.35**	<0.001	-0.08	0.285
Lat.*Lat.*Lat	**0.38**	<0.001	**0.81**	<0.001	-0.12	0.124	**0.25**	<0.001
Long.*Long.*Long.	**0.71**	<0.001	**-0.48**	<0.001	**-0.34**	<0.001	-0.10	0.227

Nominal (P values)	PCA1	PCA2	PCA3	PCA4
Environment				
Stream	0.355	0.590	<0.001	0.735
Timber	0.330	**0.035**	0.078	0.102
Bare	0.027	**<0.001**	0.288	**<0.001**

TABLE 3. Locations and environmental scores for all sites used in analysis. Regional grouping of sites: Rom & Aug, Romincka; Roz & Pol, Roztocze; Rad, Kol & Tra, Kaszuby; Mus, Mys, Bar, Ksi, Mly, Mil & Sto, Sudetes; Bia, Białowieża; Kuz & Ugo, Ukraine; Bie, San, Prz, Ust, Kom, Rym & Gor, S.E. Poland; Pod & Pie, Pieniny; Hcr, Holy Cross; Ojc, Ojców. Climatic data from Hijmans et al. (2005).

	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Rom1	0	0	0	1	2	0	0	0	1	0	2	1	1	1	1	0	0	150	4	5	1	6.1	21.5	-8.4	659	31	54.343	22.498
Rom2	0	0	0	0	1	0	0	0	1	0	2	0	1	0	1	1	1	150	1	6	2	6.1	21.6	-8.3	655	31	54.34	22.512
Rom3	0	0	0	0	1	0	0	0	0	0	2	0	0	0	2	2	0	150	1	7	0	6.1	21.6	-8.3	655	31	54.342	22.519
Rom4	0	0	0	1	2	1	0	0	1	0	2	2	1	1	1	1	0	150	5	7	1	6.2	21.6	-8.3	645	32	54.33	22.548
Rom5	0	0	0	1	1	1	1	0	0	0	2	2	1	1	2	1	0	150	4	8	0	6.2	21.7	-8.2	645	32	54.34	22.545
Rom6	0	0	0	1	1	0	0	0	0	0	1	2	0	1	1	0	0	150	4	3	0	6.3	21.7	-8.1	643	32	54.338	22.568
Rom7	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	210	1	5	1	5.8	21.5	-8.9	665	31	54.159	22.351
Rom8	0	0	2	1	0	1	0	0	1	0	2	0	0	0	0	2	0	210	0	6	1	6.0	21.7	-8.7	659	31	54.156	22.351
Rom9	0	0	1	1	1	0	0	0	1	0	0	1	0	0	1	0	1	160	2	2	2	5.9	21.3	-8.4	643	32	54.331	22.704
Rom10	0	0	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	160	2	4	1	6.1	21.6	-8.2	637	32	54.338	22.686
Aug1	0	0	0	1	2	1	0	0	1	0	1	2	1	1	1	2	1	125	5	7	2	6.5	22.3	-7.9	580	33	53.799	23.236
Aug2	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	125	2	2	1	6.5	22.4	-8.0	580	33	53.771	23.177
Roz1	0	0	2	1	0	0	1	0	1	2	1	0	0	0	0	2	0	260	0	4	3	7.1	22.8	-8.2	580	41	50.704	22.916
Roz2	0	0	1	0	0	0	1	0	2	2	0	0	0	0	0	0	1	290	0	1	5	7.2	22.9	-8.1	579	41	50.594	22.959
Roz3	0	0	0	0	2	0	0	0	1	1	0	1	0	1	0	0	1	250	4	1	3	7.3	22.8	-8.0	583	41	50.559	23.008
Roz4	1	1	2	1	1	0	0	2	0	0	1	2	0	1	0	0	1	260	4	4	1	7.3	22.8	-7.6	588	40	50.441	23.108
Roz5	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	1	1	260	0	5	2	7.0	22.5	-8.3	586	41	50.647	23.027
Roz6	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	1	1	280	0	5	2	7.0	22.6	-8.2	586	41	50.646	23.021
Roz7	0	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1	0	290	0	5	2	6.7	22.1	-8.3	606	41	50.573	23.053
Roz8	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	1	1	300	0	2	3	7.2	22.7	-8.0	583	41	50.584	23.052

(continues)

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	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Roz9	0	0	1	0	1	0	1	1	0	2	1	1	0	0	0	1	0	250	2	3	2	7.0	22.7	-8.2	570	40	50.821	23.33
Roz10	0	0	1	0	0	0	0	0	1	2	1	0	0	0	0	1	0	280	0	3	3	6.9	22.3	-8.1	600	40	50.558	23.119
Pol1	0	0	0	0	1	0	0	1	1	0	1	2	1	0	1	0	0	180	3	4	1	7.5	23.9	-7.9	515	37	51.334	23.368
Pol2	0	0	0	0	2	0	1	0	0	0	0	1	1	1	0	1	0	175	4	3	0	7.5	23.9	-7.8	518	37	51.357	23.261
Pol3	0	0	0	0	1	0	0	0	0	0	2	1	2	0	1	1	1	165	2	7	1	7.5	23.9	-7.8	525	38	51.422	23.077
Rad1	0	0	1	1	1	0	0	0	1	0	2	1	0	0	2	1	1	150	2	6	2	6.0	19.9	-5.9	592	32	54.307	18.308
Rad2	0	0	0	1	1	1	0	0	1	1	2	1	0	0	2	1	0	150	2	7	2	6.0	19.9	-5.9	592	32	54.304	18.305
Rad3	0	0	0	1	1	1	0	0	1	1	2	1	0	0	2	1	0	150	2	7	2	6.0	19.9	-5.8	592	32	54.311	18.311
Rad4	0	0	0	1	1	1	0	0	1	1	1	1	0	0	0	1	1	150	2	4	3	5.9	19.8	-6.0	593	32	54.32	18.322
Rad5	0	0	0	1	1	0	0	0	1	0	2	0	0	0	1	1	1	150	1	5	2	6.0	19.9	-5.9	592	32	54.301	18.301
Rad6	0	0	0	1	0	1	1	0	0	0	2	1	0	0	1	1	0	150	1	6	0	5.9	19.8	-6.1	600	31	54.3	18.293
Ko1	0	0	0	1	1	0	0	0	1	1	2	1	0	0	1	2	0	100	2	6	2	6.1	19.9	-5.5	564	33	54.276	18.446
Kol2	0	0	0	1	1	0	0	1	1	1	2	1	0	0	2	2	0	100	2	7	2	5.9	19.7	-5.7	575	33	54.273	18.439
Kol3	0	0	0	1	1	1	0	0	1	1	2	1	0	0	1	2	0	100	2	7	2	6.0	19.9	-5.8	575	33	54.265	18.427
Ko4	0	0	0	1	1	0	0	0	1	1	2	2	0	0	1	2	1	100	3	6	3	6.0	19.8	-5.7	575	33	54.268	18.431
Kol5	0	0	0	1	1	1	0	0	1	2	2	1	0	0	1	2	0	100	2	7	3	5.9	19.7	-5.7	575	33	54.271	18.435
Tra1	0	0	0	1	1	0	0	1	0	1	1	2	0	0	1	1	0	80	3	4	1	6.2	20.1	-5.7	559	34	54.19	18.526
Tra2	0	0	0	1	1	1	0	1	1	0	1	0	2	0	1	1	0	80	1	7	1	6.2	20.1	-5.7	559	34	54.189	18.532
Tra3	0	0	0	1	1	1	0	1	0	1	2	0	1	0	2	1	0	80	1	8	1	6.2	20.1	-5.7	559	34	54.19	18.539
Mus1	1	1	1	0	0	1	0	0	0	0	2	1	1	0	2	2	0	270	1	11	0	7.8	22.6	-5.4	583	45	50.66	16.953
Mus2	0	1	0	1	0	0	0	0	1	0	2	1	1	0	2	1	0	270	1	8	1	7.8	22.6	-5.4	583	45	50.661	16.951
Mus3	0	1	1	1	0	1	0	0	0	0	2	1	1	0	2	2	0	270	1	10	0	7.8	22.6	-5.4	583	45	50.661	16.954

(continues)

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	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Mus4	0	1	0	1	1	0	0	0	0	0	1	2	1	0	1	1	0	270	3	6	0	7.8	22.6	-5.4	583	45	50.661	16.995
Mys1	1	0	2	1	0	0	0	0	0	0	2	0	0	0	1	1	1	270	0	6	1	7.1	22.4	-7.5	631	39	51.016	16.12
Mys2	2	0	2	1	0	1	0	0	0	0	2	0	0	0	2	2	0	270	0	10	0	7.0	22.1	-7.7	644	38	51.02	16.1
Mys3	1	0	2	1	0	0	0	0	0	0	2	0	0	0	1	1	1	270	0	6	1	7.0	22.1	-7.7	644	38	51.022	16.09
Mys4	2	0	2	1	0	0	0	0	0	0	2	0	0	0	2	1	1	270	0	8	1	7.3	22.7	-7.4	624	39	51.023	16.11
Mys5	1	0	1	1	0	1	0	0	0	0	2	0	0	0	2	1	1	270	0	8	1	7.3	22.7	-7.4	624	39	51.025	16.12
Bar1	1	0	1	0	0	0	0	0	0	0	2	0	0	0	2	0	0	300	0	6	0	7.8	22.7	-5.5	575	49	50.503	16.74
Bar2	1	0	1	1	0	1	0	0	0	0	2	0	0	0	2	1	0	250	0	8	0	7.4	22.0	-5.7	598	48	50.5	16.73
Bar3	2	0	2	0	0	0	0	0	0	0	2	0	0	0	2	1	0	250	0	8	0	7.4	22.0	-5.7	598	48	50.509	16.75
Bar4	2	0	2	0	0	0	0	1	0	0	2	0	0	0	1	0	0	250	0	6	0	7.4	22.0	-5.7	598	48	50.51	16.75
Bar5	1	0	2	1	0	1	0	1	0	0	2	1	0	0	2	0	0	250	1	7	0	7.9	22.6	-5.5	574	48	50.51	16.76
Ksi1	2	0	2	1	0	0	1	0	0	1	2	0	0	0	2	2	0	310	0	9	1	7.3	22.4	-7.0	617	42	50.845	16.291
Ksi2	2	0	2	1	0	0	1	0	0	2	1	0	0	0	1	2	0	310	0	7	2	7.3	22.4	-7.0	617	42	50.843	16.291
Ksi3	2	0	2	1	0	0	1	0	0	1	2	0	0	0	0	2	0	310	0	7	1	6.8	21.7	-7.3	649	41	50.839	16.29
Ksi4	2	0	2	1	0	0	0	0	0	1	2	0	0	0	1	2	0	310	0	8	1	6.9	21.7	-7.3	644	42	50.838	16.299
Mly1	1	0	1	1	0	0	0	0	1	0	1	0	0	0	1	0	1	780	0	4	2	4.7	18.3	-7.6	765	40	50.273	16.932
Mly2	0	0	0	1	1	0	0	0	1	0	1	0	0	1	0	0	1	740	2	2	2	4.8	18.3	-7.6	765	40	50.27	16.937
Mly3	1	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	650	2	4	2	4.3	17.7	-7.9	793	39	50.267	16.942
Mly4	0	0	0	1	0	0	1	1	1	2	1	1	0	0	0	1	2	780	1	3	5	4.6	18.2	-7.7	776	40	50.256	16.989
Mly5	1	0	1	0	1	0	1	1	1	1	1	1	0	1	0	1	1	690	3	4	3	5.4	19.1	-7.1	731	41	50.28	17.001
Mil1	2	2	0	0	0	0	0	0	0	2	0	0	0	0	2	1	0	400	0	8	2	6.6	21.6	-8.2	670	38	50.943	15.935

(continues)

(continued)

	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Mil2	2	2	1	0	0	1	0	0	0	2	1	0	1	0	2	1	0	560	0	11	2	6.1	20.7	-8.2	704	36	50.937	15.938
Mi3	1	2	1	0	0	0	1	1	0	2	1	0	1	0	0	1	0	420	0	7	2	6.7	21.7	-8.1	670	38	50.939	15.918
Mil4	2	2	2	0	0	0	1	2	0	2	1	0	0	0	0	1	0	510	0	7	2	6.0	20.5	-8.3	714	36	50.937	15.909
Mil5	2	2	2	0	0	0	1	2	0	1	1	0	0	0	2	0	0	460	0	8	1	6.3	21.1	-8.3	687	37	50.931	15.947
Mil6	1	2	1	1	1	1	0	1	0	1	2	1	1	0	1	1	0	550	2	10	1	5.8	20.1	-8.3	723	36	50.929	16.002
Sto1	2	2	2	0	0	0	0	0	0	2	1	0	0	0	2	1	0	730	0	9	2	5.2	18.9	-7.7	745	39	50.498	16.35
Sto2	1	0	2	1	0	0	0	0	1	1	2	1	0	1	1	1	0	590	2	6	2	5.4	19.3	-7.5	727	41	50.477	16.372
Sto3	0	0	0	1	2	0	0	0	0	0	0	2	0	2	0	1	0	550	6	2	0	6.3	20.5	-7.0	679	43	50.446	16.309
Sto4	0	0	0	1	1	0	0	0	0	0	2	1	0	2	1	1	0	450	4	5	0	6.6	21.0	-6.8	656	44	50.429	16.287
Bia2	0	0	0	0	0	1	1	0	2	0	1	0	0	0	0	0	2	155	0	3	4	6.9	23.2	-7.8	582	32	52.696	23.816
Bia5	0	0	0	1	1	0	0	1	1	0	1	2	1	1	1	0	0	155	4	4	1	7.0	23.3	-7.7	580	31	52.701	23.815
Bia9	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	1	1	175	0	5	2	6.9	23.1	-7.8	583	31	52.7	23.794
Bia11	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	2	170	0	2	3	6.9	23.2	-7.8	582	32	52.672	23.849
Bia12	0	0	0	1	1	0	0	0	1	0	1	2	1	1	0	0	1	160	4	3	2	7.0	23.3	-7.7	580	31	52.688	23.827
Bia13	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	1	0	170	0	4	1	6.9	23.2	-7.8	581	31	52.706	23.791
Bia16	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	2	0	165	0	6	1	7.0	23.2	-7.7	581	31	52.711	23.787
Bia1	0	0	0	0	0	0	1	1	1	0	2	0	0	0	0	1	0	165	0	4	1	7.0	23.2	-7.7	580	31	52.725	23.779
Bia4	0	0	0	1	2	0	0	0	1	0	1	2	0	1	0	1	0	157	5	3	1	7.0	23.2	-7.7	581	31	52.719	23.715
Bia10	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	1	1	162	0	5	2	6.9	23.2	-7.7	581	31	52.734	23.716
Bia14	0	0	0	1	1	0	0	0	1	0	1	2	1	1	1	1	0	150	4	5	1	7.1	23.3	-7.6	578	31	52.716	23.817
Bia15	0	0	0	1	2	0	0	0	1	0	0	2	0	1	0	0	0	150	5	1	1	7.1	23.3	-7.6	578	31	52.713	23.818

(continues)

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	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Bia3	0	0	0	1	2	0	0	0	1	0	0	2	1	1	0	0	0	150	5	2	1	7.0	23.3	-7.7	580	31	52.707	23.823
Bia17	0	0	0	0	1	0	0	0	2	0	1	0	0	1	0	0	2	175	2	2	4	6.9	23.1	-7.8	583	31	52.7	23.797
Bia6	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	1	165	0	2	2	6.9	23.2	-7.8	581	31	52.706	23.799
Bia7	0	0	0	1	1	0	0	1	1	0	1	1	1	1	0	1	0	145	3	4	1	7.0	23.3	-7.7	578	30	52.8	23.826
Bia8	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	1	0	175	0	5	1	6.9	23.2	-7.7	581	31	52.702	23.702
Kuz1	2	2	1	1	1	0	0	0	1	2	1	0	0	1	1	2	0	560	2	9	3	6.9	22.3	-8.4	770	36	47.945	24.123
Kuz2	2	2	2	1	0	0	0	0	0	2	1	0	0	1	1	1	0	530	1	8	2	6.9	22.3	-8.4	770	36	47.944	24.12
Kuz3	2	2	2	1	0	0	0	0	0	2	1	0	0	0	1	1	0	510	0	8	2	6.5	21.7	-8.7	787	36	47.942	24.116
Kuz4	2	2	1	1	0	0	1	0	0	2	1	1	0	0	1	1	0	480	1	8	2	7.3	22.8	-8.2	757	35	47.94	24.114
Kuz5	2	1	1	1	0	0	0	0	0	2	1	0	0	0	1	1	0	440	0	7	2	7.3	22.8	-8.2	757	35	47.938	24.11
Ugo1	2	1	2	1	0	0	1	0	0	2	1	0	0	0	0	0	1	550	0	5	3	5.9	20.7	-8.5	841	33	48.261	23.646
Ugo2	1	1	1	1	0	1	1	0	0	2	0	0	0	0	0	0	1	540	0	4	3	5.9	20.7	-8.5	841	33	48.259	23.642
Ugo3	1	1	1	1	0	1	1	0	0	2	0	0	0	0	0	0	1	525	0	4	3	6.7	21.9	-8.0	808	32	48.259	23.636
Ugo4	1	1	2	1	0	1	0	0	0	2	1	0	0	0	1	1	1	520	0	7	3	7.6	23.0	-7.5	780	31	48.257	23.628
Ugo5	2	2	2	1	0	0	1	0	0	2	1	0	0	0	1	1	0	500	0	8	2	7.7	23.1	-7.4	778	31	48.256	23.623
Ugo6	2	2	2	1	0	0	1	0	0	2	1	0	0	0	0	0	0	520	0	6	2	7.6	23.0	-7.5	780	31	48.258	23.63
Ugo7	1	1	2	1	0	0	1	0	0	2	0	0	0	0	0	0	0	545	0	3	2	5.9	20.7	-8.5	841	33	48.26	23.644
Bie1	0	0	2	1	0	0	1	1	0	2	1	0	0	0	0	0	0	760	0	2	2	5.9	20.9	-9.1	745	38	49.605	22.64
Bie2	0	0	2	1	0	0	1	1	0	2	1	0	0	0	0	0	0	760	0	2	2	5.3	20.2	-9.3	834	33	49.081	22.64
Bie3	0	0	0	1	2	0	0	0	0	0	0	2	1	2	0	0	0	650	6	2	0	5.9	21.0	-8.9	799	33	49.113	22.663
Bie4	0	0	0	1	1	0	0	0	1	0	2	2	1	1	0	0	0	640	4	4	1	6.0	21.1	-8.9	794	33	49.126	22.673

(continues)

(continued)

	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Bie5	0	0	2	0	0	1	1	0	0	2	1	1	0	0	2	1	0	650	1	6	2	5.5	20.5	-9.2	823	33	49.119	22.66
Bie6	0	0	2	1	0	1	1	0	0	2	0	0	0	0	1	0	0	710	0	3	2	4.8	19.7	-9.9	854	33	49.172	22.476
Bie7	0	0	0	1	2	0	0	1	1	0	1	2	0	2	0	0	0	610	6	2	1	5.8	21.1	-9.3	797	33	49.162	22.457
Bie8	0	0	0	1	2	0	0	0	1	0	1	2	0	2	0	0	0	660	6	2	1	5.6	20.7	-9.1	815	33	49.105	22.632
Bie9	0	0	1	0	1	0	1	0	0	2	1	0	0	0	1	0	0	640	1	3	2	6.0	21.1	-8.9	794	33	49.126	22.67
San1	1	0	2	1	1	0	0	0	0	2	1	0	0	0	0	1	1	200	1	4	3	7.0	23.0	-9.1	670	38	49.605	22.239
San2	0	0	1	1	1	1	0	0	0	1	2	1	0	1	0	1	0	200	3	5	1	6.7	22.6	-9.4	687	37	49.607	22.188
San3	0	0	1	0	1	0	0	1	0	0	2	0	1	1	0	1	0	210	2	5	0	6.5	22.4	-9.6	696	37	49.603	22.183
San4	0	0	2	1	1	0	0	0	0	1	1	1	1	1	0	0	1	290	3	3	2	6.3	21.9	-9.5	703	38	49.648	22.326
San5	0	0	2	1	1	0	1	0	1	1	1	0	1	1	0	1	1	250	2	4	3	6.8	22.6	-9.3	686	36	49.503	22.33
San6	0	0	1	0	1	0	0	0	0	1	2	0	1	1	0	1	1	280	2	5	2	6.7	22.5	-9.2	690	37	49.509	22.337
San7	0	0	2	1	0	0	0	0	1	1	1	0	0	1	0	0	1	350	1	2	3	6.0	21.6	-9.8	731	36	49.438	22.373
Prz1	0	0	0	1	1	0	0	1	0	1	1	1	0	1	0	0	0	350	3	2	1	6.7	22.0	-8.6	689	39	49.701	22.525
Prz2	0	0	0	1	1	0	0	1	0	1	2	1	1	1	0	1	1	400	3	5	2	6.8	21.9	-8.3	690	40	49.719	22.586
Prz3	0	0	1	1	0	1	0	1	0	0	2	1	0	0	0	0	1	250	1	4	1	7.6	22.9	-7.5	650	40	49.736	22.622
Prz4	0	0	1	1	0	0	0	0	0	1	2	0	1	0	1	2	0	200	0	7	1	7.5	22.6	-7.3	660	40	49.774	22.7
Prz5	0	0	1	1	1	0	0	0	1	1	2	1	1	1	0	1	0	200	3	5	2	8.0	23.3	-7.0	631	41	49.805	22.652
Prz6	0	0	0	1	1	0	0	0	1	1	2	1	1	1	1	1	0	220	3	6	2	7.5	22.8	-7.5	652	41	49.815	22.639
Ust1	0	0	0	1	2	0	0	1	0	0	1	2	1	2	0	1	0	400	6	4	0	6.5	21.7	-8.5	721	37	49.458	22.661
Kom1	0	0	1	1	0	0	1	0	1	1	2	0	0	0	1	1	0	410	0	5	2	5.9	21.5	-9.9	752	35	49.402	22.167
Kom2	0	0	1	0	1	1	0	1	0	0	2	1	0	1	0	1	0	500	3	5	0	6.0	21.7	-9.6	770	34	49.322	22.052

(continues)

(continued)

	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Kom3	0	0	2	1	1	1	0	1	0	1	1	1	1	2	0	0	1	500	4	4	2	5.9	21.4	-9.7	773	34	49.331	22.043
Rym1	1	0	2	1	1	0	0	0	1	1	2	0	0	1	0	0	0	380	2	4	2	6.6	22.5	-9.1	743	37	49.463	21.731
Rym2	1	0	2	1	1	0	0	0	1	1	2	1	1	1	0	1	0	380	3	6	2	6.7	22.7	-9.0	734	36	49.462	21.737
Rym3	0	0	2	1	1	1	0	2	1	1	1	2	0	2	0	0	1	480	5	3	3	6.1	21.8	-9.4	771	36	49.412	21.781
Gor1	1	0	2	0	1	0	1	1	0	1	1	1	0	0	0	0	0	400	2	3	1	6.8	22.7	-8.5	787	38	49.515	21.134
Gor2	1	0	2	1	0	0	1	1	0	1	1	1	0	0	0	0	0	400	1	3	1	6.8	22.7	-8.5	787	38	49.512	21.135
Gor3	0	0	2	1	1	0	0	1	0	1	1	1	0	1	0	0	0	400	3	2	1	6.8	22.7	-8.6	774	38	49.587	21.272
Pod1	1	0	2	0	0	1	1	0	0	2	0	0	0	0	0	1	0	650	0	4	2	6.2	21.8	-9.0	804	44	49.457	20.443
Pod2	1	0	2	0	0	1	1	0	0	2	0	0	0	0	0	1	0	570	0	4	2	6.7	22.4	-8.8	776	45	49.461	20.441
Pod3	1	0	1	0	0	0	1	0	0	2	1	0	0	0	0	2	0	520	0	5	2	6.7	22.4	-8.8	776	45	49.464	20.438
Pod4	1	0	2	1	0	1	1	0	0	2	1	0	0	0	0	1	0	440	0	5	2	7.2	23.0	-8.5	747	45	49.47	20.434
Pod5	1	0	2	1	0	0	1	0	0	2	0	0	0	0	0	0	0	440	0	2	2	7.2	23.0	-8.5	747	45	49.468	20.435
Pod6	1	0	1	1	0	1	1	0	0	2	0	0	0	1	0	0	1	440	0	3	3	7.2	23.0	-8.5	747	45	49.475	20.439
Pod7	1	0	1	1	1	0	1	0	1	2	0	0	0	1	0	0	0	450	1	2	3	6.5	22.0	-8.9	795	45	49.477	20.439
Pod8	1	0	1	1	0	0	1	0	1	2	1	0	0	0	0	1	1	450	0	4	3	6.5	22.0	-8.9	795	45	49.478	20.44
Pie1	2	2	2	0	0	1	0	0	0	1	2	0	1	0	2	2	0	470	0	13	1	6.6	22.3	-8.8	767	45	49.424	20.467
Pie2	1	2	2	0	0	0	0	0	1	1	2	0	2	0	2	2	0	470	0	12	2	6.6	22.3	-8.8	767	45	49.425	20.47
Pie3	1	2	1	0	0	0	0	0	1	0	2	0	0	0	2	2	0	470	0	10	1	6.6	22.3	-8.8	767	45	49.425	20.473
Pie4	1	2	1	0	0	0	0	1	0	0	2	0	1	0	2	2	0	470	0	11	0	6.7	22.3	-8.7	766	45	49.424	20.477
Pie5	2	2	2	0	0	0	0	1	0	0	2	0	1	0	2	2	0	470	0	12	0	6.7	22.3	-8.7	766	45	49.424	20.481
Pie6	1	2	1	1	1	0	0	1	2	2	0	0	0	1	1	0	0	470	2	5	4	7.4	23.4	-8.5	724	46	49.419	20.458

(continues)

(continued)

	rock	lime	slop	stre	wet	timb	bare	distur	coni	bee	mdw	aldwil	ash	weth	dogsm	richh	acidh	alt	wetcom	richco	poorco	MeanAnnTem	MaxTemWarMon	MinTemColMon	AnnPrec	PrecSeanCV	latitude	long
Pie7	2	2	2	1	0	0	0	1	2	0	1	0	1	1	0	0	0	490	1	7	2	7.4	23.4	-8.3	722	46	49.421	20.463
Hcr1	0	0	1	1	1	0	0	0	1	0	1	1	0	0	0	0	1	250	2	2	2	6.8	22.3	-8.6	627	40	50.983	20.98
Hcr2	0	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	380	2	1	3	6.6	22.0	-8.7	652	40	50.832	21.129
Hcr3	0	0	0	0	2	0	0	0	1	0	0	2	0	2	0	0	0	260	6	1	1	7.2	22.8	-8.6	631	41	50.746	20.654
Hcr4	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	1	200	0	3	1	7.9	23.6	-7.3	564	41	50.905	21.449
Hcr5	1	1	2	1	0	0	0	1	0	1	2	1	0	0	1	0	0	180	1	6	1	8.0	23.7	-7.3	550	42	51.005	21.538
Hcr6	1	0	1	0	1	0	0	0	1	1	1	0	0	0	0	1	1	550	1	4	3	5.8	20.9	-9.4	707	40	50.858	21.052
Ojc1	2	2	2	0	0	0	0	0	0	2	1	0	0	0	2	2	0	370	0	10	2	6.9	21.7	-7.6	766	41	50.203	19.81
Ojc2	2	2	2	0	0	0	0	0	0	2	1	0	1	0	2	2	0	390	0	11	2	6.9	21.7	-7.6	766	41	50.205	19.809
Ojc3	2	2	2	0	0	0	1	0	0	2	1	0	1	0	2	2	0	350	0	11	2	7.2	22.1	-7.5	745	41	50.188	19.826
Ojc4	2	2	2	0	0	1	1	0	0	2	1	0	0	0	2	2	0	350	0	11	2	7.2	22.1	-7.5	745	41	50.189	19.829
Ojc5	2	2	2	0	0	1	0	0	1	2	2	0	0	0	2	2	0	350	0	12	3	7.5	22.5	-7.2	715	41	50.195	19.826
Ojc6	2	2	2	0	1	0	0	0	0	2	2	0	0	1	2	2	0	400	2	11	2	6.9	21.8	-7.6	765	41	50.204	19.806
Ojc7	2	2	2	0	0	1	0	0	0	2	1	0	0	0	2	2	0	400	0	11	2	6.9	21.7	-7.6	766	41	50.201	19.811

APPENDIX 2

Species/Sites matrix and taxonomic notes

Nomenclature follows Wiktor (2004), except for asterisked species: *Acicula perpusilla* (Reinhardt, 1880), *Pyramidula pusilla* (Vallot, 1801) Syn: *P. rupestris*, *Sphyradium doliolum* (Bruguière, 1792) Syn: *Orcula doliolum, Vestia* sp.: a *Vestia* as yet undetermined, but not any of those also recorded in our samples; *Trochulus* (all species), in Wiktor (2004) as *Trichia;* the latter name has prior use elsewhere. The following species were excluded from the analyses (see text): *Oxyloma elegans, Succinea sarsi, Columella aspera, Truncatellina cylindrica, Vertigo angustior, Chondrina clienta, Oxychilus inopinatus, Cepaea vindobonensis, Helix lutescens.*

	Romincka											
	Rom 1	Rom 2	Rom 3	Rom 4	Rom 5	Rom 6	Rom 7	Rom 8	Rom 9	Rom 10	Aug 1	Aug 2
Acicula parcelineata	0	0	0	0	0	0	0	0	0	0	0	0
Acicula polita	8	0	2	0	0	0	1	4	0	0	2	4
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	23	9	0	14	6	10	0	11	0	0	5	12
Carychium tridentatum	272	84	151	0	341	41	33	42	28	43	94	178
Succinea oblonga	0	0	0	539	1	0	4	0	0	0	0	14
Succinea putris	12	6	5	0	24	23	0	2	11	5	12	23
Cochlicopa lubrica	41	49	6	139	77	142	16	20	5	21	8	8
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	6
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	38	38	14	50	14	24	0	12	14	10	24	11
Vertigo pusilla	7	31	9	6	6	2	5	10	27	5	59	34
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	17	45	15	10	2	0	8	2	10	7	36	16
Vertigo alpestris	0	17	1	64	3	1	3	6	32	0	1	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	19	2	0	64	0	9	0	0	0	2	3	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	29	18	45	66	7	0	32	7	0	7	21	15
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	0	0	0	0	0	0	0	0	0	0	0
Ena obscura	1	0	3	26	0	20	8	2	0	0	0	0
Punctum pygmaeum	124	53	159	394	365	12	49	43	42	29	330	136
Discus ruderatus	0	56	0	6	10	6	0	4	22	6	17	13
Discus rotundatus	26	32	8	5	16	0	14	20	2	22	0	0
Discus perspectivus	0	0	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	28	5	0	23	13	15	16	11	2	0	4	20
Semilimax semilimax	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Romincka											
	Rom 1	Rom 2	Rom 3	Rom 4	Rom 5	Rom 6	Rom 7	Rom 8	Rom 9	Rom 10	Aug 1	Aug 2
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	28	19	8	84	29	36	0	5	4	9	2	1
Vitrea contracta	0	0	0	0	0	0	4	0	0	0	0	0
Aegopinella pura	75	55	42	150	33	38	15	14	18	26	8	28
Aegopinella minor	27	0	9	0	0	0	11	20	0	0	0	0
Aegopinella epipedostoma	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	18	45	1	33	8	23	3	5	15	22	15	17
Nesovitrea petronella	24	54	2	20	23	0	0	0	5	19	24	2
Oxychilus alliarius	1	0	0	0	0	0	0	0	0	0	1	0
Oxychilus cellarius	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus depressus	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia rufa	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	2	0	0	17	18	3	0	0	0	0	3	4
Euconulus fulvus	29	48	8	31	6	2	3	10	11	14	26	10
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	7	36	14	27	57	12	75	85	84	90	27	27
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	39	0	55	30	0	0	12	11
Ruthenica filograna	0	0	0	59	25	18	21	3	0	3	8	0
Macrogastra ventricosa	22	31	13	54	128	57	45	124	0	0	6	11
Macrogastra latestriata	0	0	4	0	0	0	1	0	0	0	5	8
Macrogastra plicatula	15	61	4	83	43	71	60	127	69	78	44	2
Macrogastra tumida	31	20	4	0	0	0	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	1	2	0	6	133	26	19	0	12	53	0	1
Clausilia pumila	0	0	0	0	3	0	0	0	0	0	9	37
Clausilia cruciata	7	22	23	25	31	10	0	0	54	0	18	13
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Romincka											
	Rom 1	Rom 2	Rom 3	Rom 4	Rom 5	Rom 6	Rom 7	Rom 8	Rom 9	Rom 10	Aug 1	Aug 2
Laciniaria plicata	2	32	35	0	0	0	17	24	0	0	0	49
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	6	23	20	25	111	0	69	70	0	0	21	68
Bradybaena fruticum	26	3	4	33	12	51	0	0	24	2	15	26
Perforatella bidentata	34	3	3	25	58	12	0	4	0	3	37	27
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	0	0	0	0	0	0	0	0	0	0	8	0
Perforatella vicina	0	0	0	0	0	0	28	77	0	0	0	0
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	56	0	3	4	8	33	11	64	4	36	0	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	0	0	0	0	0	2	2	1	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	2	0	0	0	0	4	0	0	0	0
Arianta arbustorum	7	0	5	6	10	26	0	0	23	29	0	0
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	0	0	0	1	0	0	0	0	0	0	0	0
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea hortensis	7	1	3	7	9	8	8	9	25	14	2	14
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	0	0	0	0	1	0	0	0	0	0	0

	Roztocze												
	Roz 1	Roz 2	Roz 3	Roz 4	Roz 5	Roz 6	Roz 7	Roz 8	Roz 9	Roz 10	Pol 1	Pol 2	Pol 3
Acicula parcelineata	0	0	0	0	0	0	0	0	0	0	0	0	0
Acicula polita	19	0	35	0	5	5	2	0	0	19	8	11	0
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	0	77	0	0	0	0	62	2	0	7	35	0
Carychium tridentatum	113	17	21	33	32	62	76	85	122	130	41	22	65
Succinea oblonga	0	0	0	0	0	0	1	0	0	0	0	0	99
Succinea putris	0	0	3	2	0	0	0	0	2	0	20	1	0
Cochlicopa lubrica	0	0	53	9	0	2	3	3	7	13	2	29	33
Cochlicopa lubricella	0	0	0	0	0	0	0	0	1	0	0	0	176
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	18	12	28	6	0	3	27	9	9	10	2	2	92
Vertigo pusilla	15	32	12	26	83	127	16	3	31	60	5	9	121
Vertigo antivertigo	0	0	6	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	0	0	37	0	0	0	2	98	0	2	0	0	3
Vertigo alpestris	0	25	0	2	0	53	0	56	0	1	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	12	7	0	0	0	0	2	0	0	0	800
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	13	0
Acanthinula aculeata	16	17	14	0	49	2	4	6	0	4	17	3	5
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena obscura	0	0	0	0	0	0	3	0	2	0	0	44	47
Punctum pygmaeum	58	51	33	20	87	22	15	13	60	43	136	77	102
Discus ruderatus	0	15	0	1	72	7	0	27	6	0	0	2	0
Discus rotundatus	23	45	0	34	27	60	115	15	0	76	0	0	3
Discus perspectivus	0	0	0	0	0	7	0	0	0	0	0	0	0
Vitrina pellucida	0	1	4	20	7	15	13	2	3	22	113	23	111
Semilimax semilimax	0	0	0	0	0	0	0	0	0	0	0	0	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	12	2	11	0	0	0	2	0	0	16	12	0	3
Vitrea contracta	0	0	0	0	34	0	0	7	2	0	0	0	0
Aegopinella pura	18	9	58	0	70	8	7	2	4	65	11	8	54

(continues)

(continued)

	Roztocze												
	Roz 1	Roz 2	Roz 3	Roz 4	Roz 5	Roz 6	Roz 7	Roz 8	Roz 9	Roz 10	Pol 1	Pol 2	Pol 3
Aegopinella minor	17	4	0	0	71	4	3	0	6	2	11	34	0
Aegopinella epipedostoma	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	13	8	0	6	21	0	5	18	14	17	18	4	44
Nesovitrea petronella	0	0	0	0	0	0	0	0	2	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	0	0	0	0	2	0	0	0	0	0	0	0
Oxychilus deubeli	4	0	0	8	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus depressus	0	0	0	0	0	0	0	0	0	5	0	0	0
Daudebardia rufa	1	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	0	27	0	0	0	0	0	0	0	0	20	25
Euconulus fulvus	14	14	13	0	23	25	4	23	5	27	4	6	8
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	6	7	0	0	7	29	24	17	36	14	37	74	97
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	0	0	0	0	0	0	0	2	0
Ruthenica filograna	0	0	0	0	0	0	0	0	0	0	0	0	117
Macrogastra ventricosa	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra latestriata	4	4	0	0	20	42	43	29	0	1	0	1	0
Macrogastra plicatula	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra tumida	0	1	0	0	17	1	11	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	0	0	0	0	2	0	1	0	0	0	0	0	232
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	3	0	0	0	0	0	64	1	99	0	0	25	0
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	70	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Roztocze												
	Roz 1	Roz 2	Roz 3	Roz 4	Roz 5	Roz 6	Roz 7	Roz 8	Roz 9	Roz 10	Pol 1	Pol 2	Pol 3
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	3	0	0	0	0	3	59	9	0	0	0	65	0
Bradybaena fruticum	11	0	8	23	0	5	15	0	9	3	4	19	20
Perforatella bidentata	0	0	5	0	0	0	0	0	0	0	5	27	0
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	21	4	5	15	12	5	7	9	0	11	0	0	0
Perforatella vicina	27	0	0	27	15	0	24	0	0	22	54	0	0
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	9	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	0	0	11	0	0	0	0	27	0	1	128	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	17	0	0	3	13	0	0	0	5	0	3	37	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	0	0	0	0	0	0	0	0	0	0	0	0	0
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	5	0	0	0	0	0	0	0	0	0	0	0	0
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	2	3	0	0	0	0	9	0	0	39	0	0	0
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	0	12	0	0	0	0	0	18	23	0	24	0

	Sudetes (a)													
	Mus 1	Mus 2	Mus 3	Mus 4	Mys 1	Mys 2	Mys 3	Mys 4	Mys 5	Bar 1	Bar 2	Bar 3	Bar 4	Bar 5
Acicula parcelineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acicula polita	40	12	6	2	0	2	0	0	0	1	0	0	0	0
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium tridentatum	145	105	136	43	2	97	1	134	222	2	1	0	210	706
Succinea oblonga	0	0	0	0	0	0	0	0	0	0	0	0	3	4
Succinea putris	0	0	0	0	0	0	0	0	0	2	0	0	0	2
Cochlicopa lubrica	3	2	5	6	0	1	0	17	41	0	0	2	56	136
Cochlicopa lubricella	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	15	5	17	37	0	2	0	28	93	0	0	7	0	3
Vertigo pusilla	0	0	0	0	1	27	4	12	0	0	387	41	25	0
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	2	23	0	0	0
Vertigo substriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo alpestris	0	0	0	0	0	10	0	0	0	0	0	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	9	3	1	14	2	0	2	18	34	14	2	0	0	0
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	12	6	11	0	0	0	2	4	0	0	0	0	0	0
Ena obscura	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Punctum pygmaeum	15	13	0	2	182	101	145	4	5	192	424	142	480	38
Discus ruderatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus rotundatus	10	15	60	103	30	12	14	33	75	40	24	99	19	76
Discus perspectivus	50	49	88	27	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	2	5	6	3	6	13	16	25	31	0	5	9	23	5
Semilimax semilimax	0	0	0	0	0	2	4	2	2	0	0	12	46	29
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	2	7	24	7	0	0	0
Eucobresia nivalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	23	4	2	0	0	0	2	0	2	0	0	16	0	0
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	0	0	0	0	0	2	0	0	8	0	0	5	0	3
Vitrea contracta	0	0	0	0	2	2	0	0	0	0	0	0	0	0
Aegopinella pura	54	31	28	66	30	6	21	20	49	23	2	54	107	36

(continues)

(continued)

	Sudetes (a)													
	Mus 1	Mus 2	Mus 3	Mus 4	Mys 1	Mys 2	Mys 3	Mys 4	Mys 5	Bar 1	Bar 2	Bar 3	Bar 4	Bar 5
Aegopinella minor	9	3	6	69	11	39	9	8	15	5	5	6	14	0
Aegopinella epipedostoma	10	3	2	0	0	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	1	0	0	0	1	2	0	9	10	8	2	0	0	2
Nesovitrea petronella	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	4	3	0	1	6	2	7	5	0	17	5	20	15	0
Oxychilus deubeli	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus depressus	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Daudebardia rufa	2	1	0	0	0	1	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	0	0	0	0	0	0	0	0	0	0	0	7	5
Euconulus fulvus	3	4	3	2	0	1	2	6	10	6	32	4	9	7
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	6	4	15	20	18	11	6	8	12	3	2	12	4	10
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ruthenica filograna	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Macrogastra ventricosa	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Macrogastra latestriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra plicatula	17	10	37	18	0	0	0	12	3	0	0	0	0	0
Macrogastra tumida	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	2	3	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	2	7	40	70	0	0	0	16	3	0	0	18	4	24
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	0	0	0	2	4	9	0	0	0	0	0	0	0
Alinda biplicata	67	26	123	40	1	9	10	27	0	3	1	25	8	30
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Sudetes (a)													
	Mus 1	Mus 2	Mus 3	Mus 4	Mys 1	Mys 2	Mys 3	Mys 4	Mys 5	Bar 1	Bar 2	Bar 3	Bar 4	Bar 5
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bradybaena fruticum	4	0	4	2	0	0	4	2	2	0	0	0	0	3
Perforatella bidentata	20	3	3	15	0	0	0	0	0	0	0	0	0	3
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	8	7	20	13	9	6	25	24	7	14	13	47	26	3
Perforatella vicina	12	5	7	1	0	0	0	0	0	0	2	0	7	6
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	5	2	8	0	0	0	0	0	0	0	0	0	7	7
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	10	0	0
Euomphalia strigella	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicodonta obvoluta	8	4	7	0	0	0	0	0	0	0	1	0	0	0
Helicigona lapicida	0	0	0	0	0	2	5	0	0	0	1	2	3	0
Arianta arbustorum	6	0	5	2	2	3	0	0	0	5	4	2	3	2
Causa holosericum	0	1	0	0	0	0	0	0	0	4	7	2	0	0
Chilostoma faustinum	0	0	0	0	0	0	0	0	0	0	0	0	4	0
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	0	0	0	0	18	18	11	21	7	10	4	18	4	7
Cepaea hortensis	3	3	4	1	0	3	4	0	0	0	0	0	4	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Helix pomatia	4	1	1	0	0	0	3	0	0	0	0	0	0	0

	Sudetes (b)																		
	Ksi 1	Ksi 2	Ksi 3	Ksi 4	Mly 1	Mly 2	Mly 3	Mly 4	Mly 5	Mil 1	Mil 2	Mil 3	Mil 4	Mil 5	Mil 6	Sto 1	Sto 2	Sto 3	Sto 4
Acicula parcelineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acicula polita	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	0	0	0	0	0	0	0	0	11	0	0	0	0	8	0	0	44	8
Carychium tridentatum	55	47	52	101	110	23	10	130	72	300	11	0	2	0	32	85	28	8	44
Succinea oblonga	2	9	3	4	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0
Succinea putris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Cochlicopa lubrica	5	7	5	9	19	2	7	3	5	2	4	0	0	0	2	0	19	6	25
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	17	21	22	17	8	21	18	20	9	0	3	0	0	2	9	0	27	22	12
Vertigo pusilla	5	17	13	151	64	6	17	2	1	5	5	0	1	0	0	3	20	0	0
Vertigo antivertigo	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	0	0	0	0	16	10	0	0	4	0	0	0	0	0	0	0	3	0	2
Vertigo alpestris	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	4	22	16	11	10	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	8	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	8	9	5	9	18	5	9	20	8	5	6	0	0	0	0	0	43	60	4
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	2	5	4	3	0	0	0	0	0	5	7	12	21	31	34	2	14	0	3
Ena obscura	2	3	2	3	0	0	0	0	0	1	5	9	3	17	18	0	0	0	0
Punctum pygmaeum	45	28	26	48	33	28	90	131	35	152	5	7	0	2	4	20	55	10	4
Discus ruderatus	0	0	0	0	0	0	0	8	2	0	0	0	0	0	0	18	11	0	0
Discus rotundatus	65	33	31	45	51	6	6	25	16	40	3	23	32	42	34	21	44	29	4
Discus perspectivus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	8	15	16	3	9	0	21	1	2	7	5	0	0	4	0	10	57	5	15
Semilimax semilimax	4	8	4	2	0	2	3	5	3	0	6	5	4	1	1	0	0	0	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	3	14	5	6	0	0	0	0	1	6	0	0	0	0	0	0	3	4	3
Eucobresia nivalis	0	0	0	0	9	2	0	2	3	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	4	3	4	4	12	13	0	25	23	0	0	0	0	1	0	4	8	0	0
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	0	0	0	0	0	10	1	0	0	0	0	0	0	0	0	7	2	25	5
Vitrea contracta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella pura	9	2	6	8	47	36	14	31	38	12	5	0	2	3	0	26	13	16	8

(continues)

(continued)

	Sudetes (b)																		
	Ksi 1	Ksi 2	Ksi 3	Ksi 4	Mly 1	Mly 2	Mly 3	Mly 4	Mly 5	Mil 1	Mil 2	Mil 3	Mil 4	Mil 5	Mil 6	Sto 1	Sto 2	Sto 3	Sto 4
Aegopinella minor	10	2	3	6	21	0	0	4	4	0	19	10	0	0	2	6	3	5	2
Aegopinella epipedostoma	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	2	3	2	6	19	10	3	30	22	2	1	4	0	1	0	2	15	79	8
Nesovitrea petronella	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	6	3	2	3	0	0	0	0	0	18	4	12	0	0	14	0	0	0	0
Oxychilus deubeli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	2	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	2	0	0	0
Oxychilus depressus	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Daudebardia rufa	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	3	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Euconulus fulvus	11	9	7	41	45	31	3	26	24	6	0	0	0	0	0	2	14	15	2
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	79	53	63	82	0	0	0	8	0	13	17	16	11	25	131	12	36	0	16
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ruthenica filograna	6	0	0	3	3	7	19	0	0	0	0	0	0	0	0	4	8	0	7
Macrogastra ventricosa	0	0	0	0	4	11	4	2	0	0	0	0	0	0	0	0	13	7	11
Macrogastra latestriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Macrogastra plicatula	58	32	35	144	0	22	3	7	29	35	0	3	6	0	19	43	89	0	25
Macrogastra tumida	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	40	0	0	1	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	0	23	0	0
Clausilia cruciata	0	0	0	0	20	3	4	5	13	0	0	0	0	0	0	5	13	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	3	4	6	2	0	0	0	0	0	31	0	14	17	36	10	22	0	0	0
Alinda biplicata	27	17	16	47	7	5	10	2	9	14	41	12	7	46	29	0	36	0	29
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Sudetes (b)																		
	Ksi 1	Ksi 2	Ksi 3	Ksi 4	Mly 1	Mly 2	Mly 3	Mly 4	Mly 5	Mil 1	Mil 2	Mil 3	Mil 4	Mil 5	Mil 6	Sto 1	Sto 2	Sto 3	Sto 4
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bradybaena fruticum	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	38	12	9	34	15	9	6	2	12	5	33	60	36	46	53	14	20	13	7
Perforatella vicina	8	4	6	5	0	0	0	0	0	10	10	17	0	0	4	0	0	0	47
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	3	2	0	0	0	2	12	2	2	0	0	0	13	19	0	0	3	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicodonta obvoluta	50	5	6	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	5	0	4	14	0	0	0	0	0	0	4	3	1	39	0	3	0	0	0
Arianta arbustorum	19	4	2	9	6	3	2	4	16	9	11	0	8	2	44	5	8	18	34
Causa holosericum	3	8	12	35	1	0	0	1	0	0	0	0	0	0	0	12	0	0	1
Chilostoma faustinum	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	12	8	5	5	0	0	0	0	0	4	3	6	3	0	0	12	0	0	3
Cepaea hortensis	0	0	0	2	0	0	0	0	0	0	8	3	1	23	2	0	0	0	3
Cepaea nemoralis	5	2	3	7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Helix pomatia	3	4	2	4	0	0	0	0	0	0	2	5	1	23	10	4	3	3	2

	Białowieża																
	Bia 2	Bia 5	Bia 9	Bia 11	Bia 12	Bia 13	Bia 16	Bia 1	Bia 4	Bia 10	Bia 14	Bia 15	Bia 3	Bia 17	Bia 6	Bia 7	Bia 8
Acicula parcelineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acicula polita	0	0	0	0	0	15	2	0	0	0	0	0	0	1	0	2	0
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	269	4	14	22	61	65	18	0	0	5	1	0	13	4	0	5	0
Carychium tridentatum	4	8	0	4	11	5	2	1	0	21	1	20	24	9	12	130	15
Succinea oblonga	5	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Succinea putris	4	6	6	17	37	29	29	0	0	0	4	0	0	0	0	0	0
Cochlicopa lubrica	45	30	15	15	47	31	15	0	4	6	4	0	4	8	8	19	6
Cochlicopa lubricella	0	0	0	3	0	0	2	0	0	0	0	0	0	2	0	8	0
Cochlicopa nitens	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	16	2	5	1	11	6	11	0	1	0	2	4	0	2	3	3	1
Vertigo pusilla	5	0	3	6	1	3	14	0	2	1	0	2	2	1	3	7	9
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	59	0	7	0	6	3	3	1	2	0	1	2	0	3	0	6	0
Vertigo alpestris	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	5	0	14	11	0	0	0	0	3	4	1	0	0	0
Vallonia pulchella	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	12	1	8	0	3	19	3	0	1	0	0	0	3	3	3	18	2
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena obscura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Punctum pygmaeum	108	13	13	6	25	24	23	3	18	5	3	28	21	16	21	105	23
Discus ruderatus	4	7	4	2	0	3	4	7	22	7	39	29	27	38	38	7	6
Discus rotundatus	1	6	12	0	0	0	0	17	22	21	26	43	36	16	22	27	24
Discus perspectivus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	2	2	1	2	2	0	4	1	1	0	0	0	2	2	1	8	3
Semilimax semilimax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	55	11	18	12	9	22	10	2	0	0	5	7	27	4	10	15	3
Vitrea contracta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella pura	18	9	37	5	6	31	4	0	3	2	0	2	12	8	5	14	5

(continues)

(continued)

	Białowieża																
	Bia 2	Bia 5	Bia 9	Bia 11	Bia 12	Bia 13	Bia 16	Bia 1	Bia 4	Bia 10	Bia 14	Bia 15	Bia 3	Bia 17	Bia 6	Bia 7	Bia 8
Aegopinella minor	0	2	0	0	0	1	0	0	1	0	0	1	1	0	0	8	2
Aegopinella epipedostoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	40	6	31	3	4	7	5	36	42	11	9	26	40	18	5	9	6
Nesovitrea petronella	27	24	14	6	9	15	4	31	25	5	11	10	11	16	18	11	5
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus depressus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia rufa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	1	1	6	6	46	11	4	0	0	2	13	0	3	8	1	0	0
Euconulus fulvus	18	4	4	5	3	5	1	22	21	2	7	10	11	8	6	20	5
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	6	6	6	13	8	4	26	11	6	8	4	20	25	20	14	18	7
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	0	2	0	0	1	0	2	0	5	5	2	3	1
Ruthenica filograna	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0
Macrogastra ventricosa	0	0	1	2	3	0	3	0	0	0	0	0	1	1	1	2	2
Macrogastra latestriata	0	0	0	0	0	0	0	0	2	0	0	0	4	0	0	7	0
Macrogastra plicatula	3	7	6	19	1	9	1	5	0	20	14	10	33	31	15	8	3
Macrogastra tumida	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1
Clausilia pumila	0	0	0	0	3	0	4	0	0	0	0	0	0	1	0	1	0
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	0	1	0	0	1	2	0	3	5	14	2	7	9	10	13	5
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Vestia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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(continued)

	Białowieża																
	Bia 2	Bia 5	Bia 9	Bia 11	Bia 12	Bia 13	Bia 16	Bia 1	Bia 4	Bia 10	Bia 14	Bia 15	Bia 3	Bia 17	Bia 6	Bia 7	Bia 8
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	0	0	0	0	0	0	4	0	2	1	1	0	2	4	7	5	5
Bradybaena fruticum	6	6	8	11	9	14	25	1	0	0	2	2	3	2	3	4	1
Perforatella bidentata	14	17	14	11	16	15	19	0	0	2	0	0	8	4	9	6	6
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella vicina	3	0	4	21	6	24	6	0	1	2	3	5	5	7	9	9	19
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	5	0	20	0	0	0	0	0	0	0	0	2	0	0	1	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	0	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	2	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0

	Ukraine											
	Kuz 1	Kuz 2	Kuz 3	Kuz 4	Kuz 5	Ugo 1	Ugo 2	Ugo 3	Ugo 4	Ugo 5	Ugo 6	Ugo 7
Acicula parcelineata	0	30	10	19	7	2	2	0	4	0	2	2
Acicula polita	2	5	4	12	8	2	2	0	0	1	3	3
**Acicula perpusilla*	0	4	2	5	1	0	0	0	0	0	0	0
Carychium minimum	0	0	0	0	0	0	0	0	0	0	0	0
Carychium tridentatum	145	532	107	74	87	152	148	4	31	7	19	20
Succinea oblonga	0	0	0	0	0	0	0	0	0	0	0	0
Succinea putris	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa lubrica	0	1	2	0	0	0	0	0	0	0	0	1
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	3	0	0	0	0	3	2	0	0	0	0	0
Vertigo pusilla	7	2	0	0	0	0	4	0	0	0	0	0
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	0	2	0	0	0	2	0	0	0	0	0	0
Vertigo alpestris	0	0	0	0	0	0	0	0	0	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	2	0	2	0	7	2	7	0	0	3	2
Vallonia costata	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	8	8	32	14	7	0	11	0	3	2	2	4
**Mastus bielzi*	5	3	9	1	5	1	0	0	0	10	0	0
Ena montana	0	0	0	0	0	0	0	0	0	0	0	0
Ena obscura	0	0	0	0	0	0	0	0	0	0	0	0
Punctum pygmaeum	4	30	19	37	11	14	15	6	3	12	8	10
Discus ruderatus	0	0	0	0	0	0	0	0	0	0	0	0
Discus rotundatus	0	0	0	0	0	0	0	0	0	0	0	0
Discus perspectivus	0	0	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	3	0	0	0	0	0	0	0	0	0	0	0
Semilimax semilimax	0	0	0	0	0	0	0	0	0	0	0	0
Semilimax kotulae	0	0	0	0	0	2	1	1	2	0	2	2
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	0	0	0	1	0	0	0	0	0	0	0	0
Vitrea diaphana	7	12	17	27	13	17	12	8	8	8	5	27
Vitrea transsylvanica	2	0	1	0	6	2	5	0	4	5	8	0
Vitrea crystallina	0	0	0	0	0	0	0	0	0	0	0	3
Vitrea contracta	0	2	0	4	0	1	0	0	0	0	2	0
Aegopinella pura	5	10	4	3	7	25	0	0	8	7	11	2

(continues)

(continued)

	Ukraine											
	Kuz 1	Kuz 2	Kuz 3	Kuz 4	Kuz 5	Ugo 1	Ugo 2	Ugo 3	Ugo 4	Ugo 5	Ugo 6	Ugo 7
Aegopinella minor	12	11	34	23	14	14	6	0	8	5	11	5
Aegopinella epipedostoma	8	8	2	4	15	8	5	0	15	4	8	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea petronella	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	18	7	5	3	14	19	0	9	4	4	6	4
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus depressus	2	3	1	1	5	0	16	0	4	1	5	0
Daudebardia rufa	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	2	6	6	5	5	0	1	1	2	0	3	1
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	0	0	0	0	0	0	0	0	0	0	0
Euconulus fulvus	0	3	0	0	1	2	1	1	0	4	2	0
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	0	0	1	0	0	0	3	0	3	0	9	3
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	3	1	4	2	8	3	5	0	18	9	2
Ruthenica filograna	0	10	8	21	2	0	0	0	0	21	0	1
Macrogastra ventricosa	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra latestriata	1	1	0	0	0	6	39	4	30	3	9	0
Macrogastra plicatula	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra tumida	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	0	0	0	0	0	0	0	0	0	0	0
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	14	0	3	4	2	7	0	3	10	7	17	0
Balea stabilis	0	0	0	0	0	0	6	6	0	0	0	8
**Vestia* sp.	25	3	14	8	15	16	0	5	0	12	8	6

(continues)

(continued)

	Ukraine											
	Kuz 1	Kuz 2	Kuz 3	Kuz 4	Kuz 5	Ugo 1	Ugo 2	Ugo 3	Ugo 4	Ugo 5	Ugo 6	Ugo 7
Vestia gulo	26	6	12	2	3	8	15	0	3	6	22	14
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	0	0	0	2	0	0	0	0	0	0	0	0
Bradybaena fruticum	3	0	0	1	2	0	0	0	0	0	0	0
Perforatella bidentata	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella dibothrion	0	0	3	0	0	6	3	0	6	13	5	2
Perforatella incarnata	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella vicina	13	17	10	3	7	19	31	4	5	11	24	5
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	6	0	1	3	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	0	0	0	0	0	0	0	0	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	0	0	0	0	0	0	2	0	0	0	0	1
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	4	32	23	9	9	25	21	4	2	19	6	9
Drobacia banaticum	8	8	4	2	9	0	0	0	0	0	0	0
Isognomostoma isognomostoma	22	9	13	6	11	29	37	9	30	17	22	13
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	1	4	2	1	0	0	0	0	0	1	0	0

	S.E. Poland (a)															
	Bie 1	Bie 2	Bie 3	Bie 4	Bie 5	Bie 6	Bie 7	Bie 8	Bie 9	San 1	San 2	San 3	San 4	San 5	San 6	San 7
Acicula parcelineata	0	0	0	1	1	6	0	0	0	0	0	0	1	1	1	1
Acicula polita	0	0	0	0	0	0	0	0	1	0	0	3	0	4	1	1
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	0	34	5	0	0	0	0	0	0	6	0	0	0	2	0
Carychium tridentatum	13	16	120	15	32	269	5	56	88	33	17	8	3	41	12	61
Succinea oblonga	0	0	11	5	0	0	0	42	0	0	5	0	0	15	0	16
Succinea putris	0	0	64	5	0	0	0	14	0	0	5	3	11	0	0	0
Cochlicopa lubrica	0	0	15	1	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	11	7	64	13	4	17	5	135	1	5	0	0	13	2	0	4
Vertigo pusilla	0	6	10	10	5	10	13	2	2	2	0	0	33	1	2	6
Vertigo antivertigo	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	0	15	1	0	0	3	0	26	0	0	0	0	0	0	1	0
Vertigo alpestris	0	0	0	3	0	1	0	0	2	0	0	0	0	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	3	2	0	14	44	19	5	20	10	16	8	4	17	6	22	5
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	1	23	2	12	2	34	8	0	0	10	0	9	11	11	4
Ena obscura	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0
Punctum pygmaeum	136	46	85	16	165	262	19	48	85	46	27	23	51	6	42	45
Discus ruderatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus rotundatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus perspectivus	0	0	21	39	13	70	39	53	23	14	80	3	18	35	32	13
Vitrina pellucida	0	0	25	28	7	4	10	121	1	0	0	0	0	0	0	0
Semilimax semilimax	3	4	6	8	10	7	1	0	7	2	0	0	2	2	3	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	2	7	0	8	4	5	3	5	0	0	0	3	0	0	0	0
Vitrea diaphana	1	0	2	5	5	24	16	2	5	77	33	17	11	32	52	49
Vitrea transsylvanica	5	4	9	7	5	7	2	3	8	0	0	0	0	0	0	0
Vitrea crystallina	0	0	3	0	0	29	0	7	2	0	0	0	0	1	0	0
Vitrea contracta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella pura	25	27	20	17	42	60	21	52	33	35	11	19	5	3	10	17

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	S.E. Poland (a)															
	Bie 1	Bie 2	Bie 3	Bie 4	Bie 5	Bie 6	Bie 7	Bie 8	Bie 9	San 1	San 2	San 3	San 4	San 5	San 6	San 7
Aegopinella minor	0	0	0	0	0	0	0	0	0	19	2	23	20	12	32	68
Aegopinella epipedostoma	4	8	4	16	41	22	32	56	15	4	3	3	3	4	12	34
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Nesovitrea petronella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	5	0	1	18	2	9	5	2	0	10	2	7	0	6	2	2
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	1	0	0	1	0	3	4	2	0	17	2	0	1
Oxychilus depressus	0	2	0	2	0	4	0	0	0	0	0	6	0	0	1	1
Daudebardia rufa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
Carpathica calophana	2	0	0	0	0	0	2	0	1	3	0	0	0	0	0	0
Zonitoides nitidus	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Euconulus fulvus	9	16	45	2	30	6	3	3	3	0	0	0	0	0	0	0
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	0	0	0	0	0	0	0	0	0	5	15	3	1	0	0	3
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	1	1	1	2	3	0	3	11	0	2	0	0	0	0	0
Ruthenica filograna	0	0	0	2	3	0	52	0	15	0	32	61	0	0	0	0
Macrogastra ventricosa	0	0	0	0	0	0	1	19	0	0	0	0	0	0	0	0
Macrogastra latestriata	10	49	10	10	7	38	2	0	8	0	0	0	19	0	0	0
Macrogastra plicatula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra tumida	8	4	72	37	14	22	44	51	7	73	36	85	46	23	59	92
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia cruciata	0	0	0	4	5	0	0	2	4	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	0	0	0	0	0	0	0	0	2	4	0	5	7	5	0
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	6	13	2	23	0	4	0	11	37	0	6	32	2
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

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	S.E. Poland (a)															
	Bie 1	Bie 2	Bie 3	Bie 4	Bie 5	Bie 6	Bie 7	Bie 8	Bie 9	San 1	San 2	San 3	San 4	San 5	San 6	San 7
Vestia gulo	13	2	38	16	3	9	2	8	2	57	15	73	3	9	7	25
Vestia turgida	0	0	39	8	3	0	13	5	1	14	12	0	21	0	8	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	0	2	1	2	0	5	2	4	5	0	25	12	15	28	0	4
Bradybaena fruticum	0	0	90	0	0	0	6	9	0	0	5	31	0	20	0	2
Perforatella bidentata	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella dibothrion	12	0	0	12	15	3	22	8	12	10	39	4	3	1	2	8
Perforatella incarnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella vicina	20	5	18	20	29	7	2	22	28	0	5	0	15	7	6	2
Perforatella umbrosa	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	41	0	0	15	0	60	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	10	3	0	18	0	0	14	12	15	1	0	17	9
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	0	0	0	0	0	0	0	0	0	6	9	0	0	7	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	1	0	18	3	7	4	3	0	0	2	8	22	0	2	0	2
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	9	0	7	8	9	13	5	0	17	9	3	4	15	25	6	12
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	10	5	19	8	26	27	0	32	6	13	8	35	8	35	15	9
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	0	0	4	0	0	0	0	0	17	19	4	1	3	7	3

	S.E. Poland (b)															
	Prz 1	Prz 2	Prz 3	Prz 4	Prz 5	Prz 6	Ust 1	Kom 1	Kom 2	Kom 3	Rym 1	Rym 2	Rym 3	Gor 1	Gor 2	Gor 3
Acicula parcelineata	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0
Acicula polita	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	3	0	0	0	0	25	0	0	87	6	0	0	0	0	0
Carychium tridentatum	23	19	301	28	104	46	59	47	118	215	56	54	7	0	0	0
Succinea oblonga	0	0	1	0	5	4	0	0	0	0	2	12	3	0	0	0
Succinea putris	0	0	2	0	6	0	81	0	2	35	3	2	0	4	0	23
Cochlicopa lubrica	0	2	2	0	0	5	0	0	5	1	118	5	0	0	0	0
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	49	6	6	0	18	2	11	5	7	17	17	3	54	3	0	7
Vertigo pusilla	0	37	1	0	3	3	24	4	17	13	7	5	47	23	21	0
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Vertigo substriata	0	0	0	0	0	0	0	0	0	18	0	0	5	0	0	0
Vertigo alpestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0
Acanthinula aculeata	1	2	11	0	19	32	2	15	13	23	3	21	9	2	6	0
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	0	0	0	0	0	28	10	5	0	30	13	0	0	0	0
Ena obscura	0	3	0	1	3	2	0	4	0	2	0	0	0	0	0	0
Punctum pygmaeum	8	2	2	3	47	44	5	59	93	44	22	141	21	21	12	3
Discus ruderatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus rotundatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus perspectivus	5	6	92	9	0	22	11	58	4	3	46	18	7	0	4	0
Vitrina pellucida	14	7	3	0	0	1	1	2	2	21	1	12	60	11	12	3
Semilimax semilimax	1	0	0	0	0	4	1	3	8	5	0	0	0	0	0	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	2	0	0	0	0	0	0	0	0	0	1	4	2	0	0	1
Vitrea diaphana	2	0	9	7	14	23	0	14	0	2	76	9	13	18	11	3
Vitrea transsylvanica	0	0	0	1	0	0	0	0	0	2	5	2	3	0	0	0
Vitrea crystallina	0	0	0	2	9	9	0	2	1	0	10	0	0	0	0	0
Vitrea contracta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella pura	7	5	3	11	3	29	5	13	22	13	13	61	16	17	13	0

(continues)

(continued)

	S.E. Poland (b)															
	Prz 1	Prz 2	Prz 3	Prz 4	Prz 5	Prz 6	Ust 1	Kom 1	Kom 2	Kom 3	Rym 1	Rym 2	Rym 3	Gor 1	Gor 2	Gor 3
Aegopinella minor	10	9	4	4	3	27	11	8	24	0	17	16	11	7	6	8
Aegopinella epipedostoma	6	0	10	0	27	8	2	4	3	0	17	4	3	0	3	0
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	0	0	0	0	0	0	0	0	0	23	0	0	3	6	0	2
Nesovitrea petronella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	4	0	6	0	0	25	2	11	3	0	5	0	4	18	12	6
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	4	0	6	6	0	0	1	2	9	4	2	8	5	2
Oxychilus depressus	0	0	0	0	0	2	0	0	0	0	3	0	0	0	0	0
Daudebardia rufa	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	4	0	0	0	0	5	0	0	20	56	2	0	0	0	0
Euconulus fulvus	1	3	0	0	0	3	0	0	0	0	5	2	4	0	0	13
Euconulus alderi	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
Cochlodina laminata	0	16	9	79	32	8	0	1	10	9	13	8	0	6	0	8
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	0	0	0	0	2	0	3	4	0	2	5	18
Ruthenica filograna	0	0	0	0	0	0	0	0	0	0	4	15	0	0	0	0
Macrogastra ventricosa	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra latestriata	0	16	16	0	6	12	0	14	9	0	12	10	6	10	0	10
Macrogastra plicatula	0	0	0	0	0	0	0	0	0	0	10	13	0	0	9	0
Macrogastra tumida	65	38	74	29	7	23	21	51	90	2	40	12	5	3	0	12
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia pumila	0	0	28	0	65	0	0	0	0	0	0	0	0	0	0	0
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	20	9	22	15	20	0	6	10	0	18	7	0	0	0	0
Alinda biplicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	27	9	0	6	0	16	4	20	0	0	0	0	0	10	7	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	S.E. Poland (b)															
	Prz 1	Prz 2	Prz 3	Prz 4	Prz 5	Prz 6	Ust 1	Kom 1	Kom 2	Kom 3	Rym 1	Rym 2	Rym 3	Gor 1	Gor 2	Gor 3
Vestia gulo	12	4	12	6	25	25	10	19	31	12	36	17	10	5	11	49
Vestia turgida	0	0	0	36	0	0	0	0	0	0	144	16	17	6	5	0
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	7	11	39	4	0	19	4	25	13	5	0	0	0	7	7	15
Bradybaena fruticum	0	0	70	78	93	17	55	7	0	1	10	10	0	16	11	0
Perforatella bidentata	0	0	0	0	23	0	0	0	0	0	84	6	2	0	0	35
Perforatella dibothrion	18	30	20	46	0	12	8	39	56	88	5	8	19	8	6	3
Perforatella incarnata	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4
Perforatella vicina	13	22	19	28	5	16	3	6	19	15	13	21	31	17	19	7
Perforatella umbrosa	0	0	0	22	21	0	0	0	0	0	0	0	0	0	0	0
Perforatella rubiginosa	0	0	0	0	0	0	2	0	0	0	39	0	0	0	0	0
**Trochulus villosulus*	0	0	2	0	37	0	0	0	0	0	0	0	0	0	0	0
**Trochulus hispidus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus lubomirskii*	31	0	0	0	0	1	0	0	6	9	0	0	0	0	0	3
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	3	8	0
**Trochulus bielzi*	0	0	9	2	10	23	5	14	0	0	0	3	0	0	0	2
**Trochulus unidentatus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euomphalia strigella	43	5	14	10	0	0	0	0	3	0	0	0	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	0	0	0	56	71	0	16	2	3	0	0	6	3	9	12	10
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	11	6	20	20	39	15	5	2	14	6	7	7	10	0	6	4
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	11	7	13	5	1	11	7	4	29	10	38	35	9	11	21	8
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	9	10	21	53	75	6	3	7	7	0	0	4	2	8	6	2

	Pieniny														
	Pod 1	Pod 2	Pod 3	Pod 4	Pod 5	Pod 6	Pod 7	Pod 8	Pie 1	Pie 2	Pie 3	Pie 4	Pie 5	Pie 6	Pie 7
Acicula parcelineata	0	0	0	0	0	0	0	0	3	3	0	3	3	0	0
Acicula polita	0	0	5	0	3	4	0	0	3	5	0	0	0	2	3
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	50	0	22	0	3	39	0	0	0	0	0	0	0	4	16
Carychium tridentatum	40	65	63	41	71	64	42	46	133	37	19	55	45	25	74
Succinea oblonga	0	0	0	0	0	0	0	0	3	0	0	0	0	0	12
Succinea putris	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
Cochlicopa lubrica	0	0	0	0	0	0	0	0	2	0	0	0	0	0	4
Cochlicopa lubricella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columella edentula	3	2	4	3	1	7	3	14	4	8	2	4	5	3	9
Vertigo pusilla	6	6	10	5	7	10	14	17	28	23	5	49	13	10	22
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	9	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo alpestris	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
**Sphyradium doliolum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Vallonia costata	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	2	7	0	36	3	5	4	8	2	8	4	5	4	0	1
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	1	0	2	0	4	0	2	19	22	6	23	57	9	16
Ena obscura	0	0	1	0	0	0	0	0	7	0	0	0	11	0	0
Punctum pygmaeum	10	45	75	34	81	43	73	33	75	60	45	21	53	133	74
Discus ruderatus	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discus rotundatus	5	3	6	5	15	1	0	0	3	11	0	4	10	0	0
Discus perspectivus	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0
Vitrina pellucida	0	0	0	0	0	3	3	3	1	6	3	4	2	1	2
Semilimax semilimax	0	0	0	0	0	0	4	0	1	2	1	0	0	0	0
Semilimax kotulae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	4	3	4	8	2	3	0	3	1	4	4	1	1	3	4
Vitrea diaphana	4	7	6	16	14	13	11	4	13	6	2	7	10	6	5
Vitrea transsylvanica	2	1	0	0	0	15	4	5	2	2	2	4	0	0	0
Vitrea crystallina	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Vitrea contracta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella pura	15	10	25	46	35	20	5	17	56	20	13	18	25	39	32

(continues)

(continued)

	Pieniny														
	Pod 1	Pod 2	Pod 3	Pod 4	Pod 5	Pod 6	Pod 7	Pod 8	Pie 1	Pie 2	Pie 3	Pie 4	Pie 5	Pie 6	Pie 7
Aegopinella minor	0	0	0	0	0	6	4	3	0	0	0	2	1	0	0
Aegopinella epipedostoma	5	5	2	4	9	3	0	1	10	12	9	8	15	2	2
Aegopinella nitidula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nesovitrea hammonis	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0
Nesovitrea petronella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus deubeli	0	0	0	3	0	4	2	0	3	9	7	10	13	1	2
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	2	0	0	0	0	5	2	5	2	4	2	1	8	0	0
Oxychilus depressus	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0
Daudebardia rufa	0	1	0	0	0	0	3	1	0	0	0	0	0	0	0
Daudebardia brevipes	0	1	0	0	0	3	1	0	0	0	0	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euconulus fulvus	0	2	4	2	4	31	42	7	15	14	7	12	1	13	2
Euconulus alderi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	7	13	2	3	6	0	0	0	4	3	2	5	6	13	5
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	2	0	0	0	2	1	0	0	0	0	0
Ruthenica filograna	0	0	0	10	1	0	0	0	31	3	0	1	11	0	0
Macrogastra ventricosa	5	8	3	0	0	7	0	4	1	3	0	0	2	12	8
Macrogastra latestriata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra plicatula	4	12	0	2	2	0	3	0	3	15	9	8	13	10	0
Macrogastra tumida	2	1	1	0	0	0	0	0	1	0	0	10	7	5	4
Clausilia parvula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia dubia	2	0	0	0	0	0	0	0	0	0	0	0	11	0	0
Clausilia pumila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	7	0	0	0	2	0	0	5	15	3	4	8	25	1	0
Alinda biplicata	17	3	2	2	5	5	4	3	3	0	5	0	6	12	0
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*Vestia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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	Pieniny														
	Pod 1	Pod 2	Pod 3	Pod 4	Pod 5	Pod 6	Pod 7	Pod 8	Pie 1	Pie 2	Pie 3	Pie 4	Pie 5	Pie 6	Pie 7
Vestia gulo	7	10	5	0	0	11	34	6	2	3	11	6	3	8	4
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Vestia elata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulgarica cana	5	3	0	0	1	5	0	4	2	7	0	0	3	0	5
Bradybaena fruticum	0	3	0	2	0	0	3	0	5	0	0	3	0	0	14
Perforatella bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	0	0	4	1	4	2	10	7	8	14	8	4	10	3	8
Perforatella vicina	3	1	6	0	2	0	0	0	4	9	7	6	16	19	2
Perforatella umbrosa	0	0	0	2	0	3	4	10	0	2	6	4	3	3	3
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	1	2	0	1	0	1	0	5	1	0	0	0
**Trochulus hispidus*	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	2	1	12	6	2	3	8	8	29	11	7	7	11	11	12
Euomphalia strigella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arianta arbustorum	0	0	0	0	0	0	0	6	3	9	6	7	10	6	26
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	7	4	2	5	5	3	6	8	11	15	11	11	29	19	4
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	11	14	5	20	8	19	19	15	35	19	5	36	25	14	27
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	2	5	7	3	2	0	0	10	3	4	1	2	12	7

	Holy Cross						Ojców						
	Hcr 1	Hcr 2	Hcr 3	Hcr 4	Hcr 5	Hcr 6	Ojc 1	Ojc 2	Ojc 3	Ojc 4	Ojc 5	Ojc 6	Ojc 7
Acicula parcelineata	0	0	0	0	0	0	1	3	6	0	2	0	2
Acicula polita	0	0	0	0	0	0	2	14	2	2	3	4	5
**Acicula perpusilla*	0	0	0	0	0	0	0	0	0	0	0	0	0
Carychium minimum	0	3	39	0	0	0	0	0	0	0	0	0	0
Carychium tridentatum	3	4	12	4	7	117	322	724	157	174	464	242	249
Succinea oblonga	0	0	0	0	1	0	0	0	0	0	0	0	0
Succinea putris	3	1	11	3	3	0	0	0	0	0	0	0	0
Cochlicopa lubrica	4	12	38	10	4	9	12	10	3	3	2	0	0
Cochlicopa lubricella	0	0	0	9	0	0	0	0	0	0	0	0	0
Cochlicopa nitens	0	0	0	0	0	0	0	0	0	0	0	0	0
**Pyramidula pusilla*	0	0	0	0	0	0	0	23	3	49	49	34	66
Columella edentula	31	4	203	28	95	44	2	19	3	6	11	12	2
Vertigo pusilla	6	0	9	4	11	4	65	13	44	27	112	26	54
Vertigo antivertigo	0	0	0	0	0	0	0	0	0	0	0	0	0
Vertigo substriata	2	2	15	0	0	2	0	0	0	0	0	0	0
Vertigo alpestris	0	0	0	0	0	1	3	2	3	6	2	3	1
**Sphyradium doliolum*	0	0	0	0	0	0	0	2	0	31	9	0	16
Pagodulina pagodula	0	0	0	0	0	0	0	0	0	0	0	0	0
Argna bielzi	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallonia costata	0	0	0	19	0	0	2	12	4	16	14	43	18
Vallonia pulchella	0	0	0	0	0	0	0	0	0	0	0	0	0
Acanthinula aculeata	6	3	0	0	18	3	2	2	2	2	0	3	7
**Mastus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0
Ena montana	0	0	0	0	0	7	4	0	5	32	10	14	12
Ena obscura	0	0	0	0	0	0	0	0	0	0	0	0	0
Punctum pygmaeum	1	5	22	140	7	3	112	123	168	143	99	124	185
Discus ruderatus	4	0	0	0	0	2	0	0	0	0	0	0	0
Discus rotundatus	4	0	0	0	0	4	32	30	6	4	23	13	3
Discus perspectivus	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrina pellucida	25	4	8	7	54	60	4	3	2	2	3	1	4
Semilimax semilimax	0	0	0	0	0	0	0	0	0	0	0	0	0
Semilimax kotulae	4	0	0	0	0	3	0	0	0	0	0	0	0
Eucobresia diaphana	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucobresia nivalis	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea diaphana	0	0	0	0	0	0	11	5	16	8	5	6	3
Vitrea transsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitrea crystallina	9	13	0	0	0	12	19	16	10	3	10	1	12
Vitrea contracta	0	0	0	0	0	0	0	4	7	9	4	2	4
Aegopinella pura	9	12	0	4	25	61	38	64	24	7	32	51	32

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(continued)

	Holy Cross						Ojców						
	Hcr 1	Hcr 2	Hcr 3	Hcr 4	Hcr 5	Hcr 6	Ojc 1	Ojc 2	Ojc 3	Ojc 4	Ojc 5	Ojc 6	Ojc 7
Aegopinella minor	0	0	0	4	14	3	3	4	4	4	3	2	6
Aegopinella epipedostoma	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegopinella nitidula	0	0	0	0	0	0	4	3	2	0	1	0	3
Nesovitrea hammonis	45	36	54	14	17	14	1	0	1	0	0	0	0
Nesovitrea petronella	11	39	0	0	0	0	0	0	0	0	0	0	0
Oxychilus alliarius	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus cellarius	0	1	0	0	0	15	0	0	0	0	0	0	0
Oxychilus deubeli	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus draparnaudi	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxychilus glaber	0	0	0	0	0	0	0	1	0	2	0	2	2
Oxychilus depressus	0	0	0	0	0	0	2	2	0	2	0	0	3
Daudebardia rufa	0	0	0	0	0	0	0	0	2	0	0	0	0
Daudebardia brevipes	0	0	0	0	0	0	1	0	1	0	0	0	0
Carpathica calophana	0	0	0	0	0	0	0	0	0	0	0	0	0
Zonitoides nitidus	8	40	49	0	13	0	0	0	0	0	0	0	0
Euconulus fulvus	11	14	7	0	4	40	31	11	10	3	6	11	5
Euconulus alderi	0	0	2	0	0	0	0	0	0	0	0	0	0
Cochlodina laminata	0	0	0	37	89	15	14	11	46	15	19	18	23
Cochlodina costata	0	0	0	0	0	0	0	0	0	0	0	0	0
Cochlodina orthostoma	0	0	0	0	0	0	5	5	4	6	3	4	29
Ruthenica filograna	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra ventricosa	0	0	0	0	0	0	1	6	4	0	13	2	3
Macrogastra latestriata	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrogastra plicatula	0	0	0	0	0	0	24	13	5	3	0	18	9
Macrogastra tumida	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia parvula	0	0	0	0	0	0	2	2	0	12	11	9	6
Clausilia dubia	0	0	0	0	0	6	11	10	3	7	17	9	28
Clausilia pumila	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia cruciata	0	0	0	0	0	0	0	0	0	0	0	0	0
Clausilia bidentata	0	0	0	0	0	0	0	0	0	0	0	0	0
Laciniaria plicata	0	0	0	18	0	0	0	0	0	0	0	0	0
Alinda biplicata	0	0	0	0	0	77	34	49	113	133	62	86	64
Balea fallax	0	0	0	0	0	0	0	0	0	0	0	0	0
Balea stabilis	0	0	0	0	0	0	0	0	0	0	0	0	0
**Vestia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	0

(continues)

(continued)

	Holy Cross						Ojców						
	Hcr 1	Hcr 2	Hcr 3	Hcr 4	Hcr 5	Hcr 6	Ojc 1	Ojc 2	Ojc 3	Ojc 4	Ojc 5	Ojc 6	Ojc 7
Vestia gulo	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia turgida	0	0	0	0	0	0	0	0	0	0	0	0	0
Vestia elata	0	0	0	0	0	134	0	0	0	0	0	0	0
Bulgarica cana	0	0	0	0	0	0	0	0	0	0	0	0	0
Bradybaena fruticum	0	0	0	65	47	0	0	0	0	0	0	0	0
Perforatella bidentata	19	0	21	0	16	0	0	0	0	0	0	0	0
Perforatella dibothrion	0	0	0	0	0	0	0	0	0	0	0	0	0
Perforatella incarnata	50	6	20	0	93	17	7	6	30	22	13	34	25
Perforatella vicina	0	0	0	0	0	0	9	3	47	38	45	23	8
Perforatella umbrosa	0	0	0	0	0	0	0	1	7	23	8	4	10
Perforatella rubiginosa	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus villosulus*	0	0	0	0	0	0	31	9	0	0	0	1	12
**Trochulus hispidus*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus lubomirskii*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bakowskii*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus bielzi*	0	0	0	0	0	0	0	0	0	0	0	0	0
**Trochulus unidentatus*	0	0	0	0	0	0	9	20	14	24	19	17	22
Euomphalia strigella	0	0	0	10	14	0	0	0	0	0	0	0	0
Helicodonta obvoluta	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicigona lapicida	0	0	0	0	0	0	1	0	2	1	2	0	1
Arianta arbustorum	0	0	0	0	38	0	0	0	0	0	0	0	0
Causa holosericum	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilostoma faustinum	0	0	0	0	0	0	14	9	26	70	42	40	64
Drobacia banaticum	0	0	0	0	0	0	0	0	0	0	0	0	0
Isognomostoma isognomostoma	0	0	0	0	0	0	25	13	18	30	23	20	13
Cepaea hortensis	0	0	0	0	0	0	0	0	0	0	0	0	2
Cepaea nemoralis	0	0	0	0	0	0	0	0	0	0	0	0	0
Helix pomatia	0	0	0	0	12	3	0	0	0	4	4	0	0

APPENDIX 3

TABLE 1. DCA of 161 sites based on all species. Spearman's correlation coefficients between explanatory variables and sites scores on the first four axes. Correlations with species richness and abundances are also shown. Values of Spearman correlation coefficient (r_s) and significance probabilities (P) are shown. Significant correlations after Bonferroni correction are in bold (the cut level was P = 0.0014). Whitney U test was used for testing relationships between nominal variables and sites scores on the DCA axes. Significant relationships (P = 0.05) are in bold. Eigenvalues: 1st axis 0.332, 2nd axis 0.132, 3rd axis 0.082, 4th axis 0.060. Percentage variance of the species data explained: 1st axis 16.9%, 2nd axis 6.7%, 3rd axis 4.2%, 4th axis 3.0%.

	DCA1		DCA2		DCA3		DCA4	
Continuous and ordinal	r_s	P	r_s	P	r_s	P	r_s	P
Environment								
Rock	**0.39**	<0.001	**-0.53**	<0.001	**0.36**	<0.001	**0.37**	<0.001
Limestone	**0.35**	<0.001	**-0.44**	<0.001	**0.27**	<0.001	0.13	0.110
Slope	**0.56**	<0.001	**-0.31**	<0.001	0.20	0.009	0.22	0.005
Wet	**-0.27**	<0.001	**0.29**	<0.001	**-0.35**	<0.001	-0.13	0.097
Disturbance	0.03	0.740	0.03	0.730	**-0.28**	<0.001	-0.03	0.714
Conifers	**-0.42**	<0.001	**0.29**	<0.001	0.04	0.642	-0.10	0.227
Beech	**0.51**	<0.001	**-0.32**	<0.001	**0.36**	<0.001	-0.04	0.597
Mixed deciduous wood	-0.11	0.159	-0.24	0.003	**-0.32**	<0.001	0.00	0.973
Alder/Willow	**-0.30**	<0.001	**0.28**	<0.001	**-0.42**	<0.001	-0.12	0.143
Ash	0.00	0.954	0.04	0.628	**-0.34**	<0.001	0.05	0.518
Wet indicator herbs	0.08	0.318	**0.40**	<0.001	**-0.25**	<0.001	0.05	0.552
Dog's mercury	-0.07	0.385	**-0.55**	<0.001	0.09	0.257	0.21	0.007
Rich indicator herbs	-0.04	0.607	**-0.51**	<0.001	0.08	0.285	0.01	0.923
Acid indicator herbs combination	-0.13	0.104	**0.30**	<0.001	0.07	0.382	-0.08	0.320
Altitude	**0.67**	<0.001	-0.08	0.311	**0.29**	<0.001	**0.42**	<0.001
Wet combination	-0.22	0.005	**0.33**	<0.001	**-0.44**	<0.001	-0.11	0.179
Rich combination	0.11	0.180	**-0.65**	<0.001	0.05	0.515	0.19	0.017
Poor combination	0.16	0.046	0.03	0.735	**0.38**	<0.001	-0.12	0.129
Climate								
Mean Annual Temp.	0.03	0.686	0.07	0.353	-0.01	0.932	-0.09	0.264
Max. Temp. Warm. Month	-0.01	0.885	**0.41**	<0.001	-0.03	0.682	-0.22	0.004
Min. Temp. Cold. Month	**-0.47**	<0.001	**-0.36**	<0.001	0.01	0.942	-0.05	0.561
Annual Precipitation	**0.74**	<0.001	**-0.09**	0.253	**0.29**	<0.001	**0.27**	<0.001
Precip. Sean. CV	**0.31**	<0.001	**-0.31**	<0.001	0.02	0.768	**0.48**	<0.001
Space								
Latitude	**-0.89**	<0.001	-0.02	0.830	-0.21	0.008	-0.13	0.089
Longitude	0.06	0.482	**0.67**	<0.001	0.18	0.025	**-0.56**	<0.001
Lat.*Long.	-0.13	0.109	**0.71**	<0.001	0.09	0.280	**-0.54**	<0.001
Lat.*Lat.	**-0.89**	<0.001	-0.02	0.830	-0.21	0.008	-0.13	0.089
Long.*Long.	0.06	0.482	**0.67**	<0.001	0.18	0.025	**-0.56**	<0.001
Lat.*Lat.*Long.	-0.24	0.002	**0.64**	<0.001	0.01	0.955	**-0.60**	<0.001
Lat.*Long.*Long.	-0.07	0.369	**0.69**	<0.001	0.13	0.114	**-0.53**	<0.001
Lat.*Lat.*Lat	**-0.89**	<0.001	-0.02	0.830	-0.21	0.008	-0.13	0.089
Long.*Long.*Long.	0.06	0.482	**0.67**	<0.001	0.18	0.025	**-0.56**	<0.001
No. Individuals	-0.09	0.242	-0.15	0.055	-0.23	0.003	0.11	0.178
No. Species	**0.34**	<0.001	-0.12	0.127	-0.11	0.162	0.15	0.061

(continues)

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Nominal (P values)	DCA1	DCA2	DCA3	DCA4
Environment				
Stream	0.238	0.515	0.143	0.068
Timber	0.437	0.154	0.799	0.910
Bare	0.011	0.556	0.001	0.915

TABLE 2. DCA of 161 sites based on large species. Spearman's correlation coefficients between explanatory variables and sites scores on the first four axes for continuous and ordinal variables. Values of Spearman correlation coefficient (r_s) and significance probabilities (P) are shown. Significant correlations after Bonferroni correction are in bold (the cut level was P = 0.0014). Whitney U test was used for testing relationships between nominal variables and sites scores on the DCA axes. Values of P< 0.05 in bold. Eigenvalues: 1st axis 0.443, 2nd axis 0.165, 3rd axis 0.101, 4th axis 0.088. Percentage variance of the species data explained: 1st axis 16.6%, 2nd axis 6.1%, 3rd axis 3.8%, 4th axis 3.3%.

	DCA1		DCA2		DCA3		DCA4	
Continuous and ordinal	r_s	P	r_s	P	r_s	P	r_s	P
Environment								
Rock	**0.36**	<0.001	**0.68**	<0.001	0.19	0.013	0.19	0.014
Limestone	**0.34**	<0.001	**0.43**	<0.001	0.15	0.056	0.17	0.027
Slope	**0.55**	<0.001	**0.42**	<0.001	0.23	0.004	0.01	0.876
Wet	**-0.27**	<0.001	**-0.39**	<0.001	**-0.37**	<0.001	-0.08	0.291
Disturbance	0.02	0.819	-0.06	0.436	-0.04	0.578	-0.24	0.003
Conifers	**-0.41**	<0.001	**-0.31**	<0.001	-0.13	0.109	0.19	0.017
Bee	**0.49**	<0.001	**0.37**	<0.001	**0.36**	<0.001	0.05	0.530
mdw	-0.17	0.030	0.05	0.564	-0.21	0.009	**-0.33**	<0.001
aldwil	**-0.29**	<0.001	**-0.42**	<0.001	**-0.40**	<0.001	-0.12	0.126
Ash	0.01	0.992	-0.14	0.078	**-0.26**	<0.001	-0.13	0.103
weth	0.12	0.127	**-0.30**	<0.001	-0.23	0.003	0.09	0.232
dogsm	-0.14	0.079	**0.46**	<0.001	-0.12	0.128	-0.05	0.570
richh	-0.11	0.156	**0.38**	<0.001	0.09	0.278	-0.13	0.089
acidh	-0.11	0.146	-0.24	0.002	0.07	0.401	0.05	0.568
Altitude	**0.69**	<0.001	**0.40**	<0.001	0.17	0.030	0.23	0.004
Wet combination	-0.22	0.006	**-0.43**	<0.001	**-0.44**	<0.001	-0.08	0.286
Rich combination	0.03	0.715	**0.53**	<0.001	-0.04	0.598	-0.09	0.254
Poor combination	0.16	0.043	0.04	0.659	**0.26**	<0.001	0.19	0.014
Climate								
Mean Annual Temp.	0.08	0.326	-0.10	0.207	0.18	0.024	-0.05	0.489
Max. Temp. Warm. Month	0.09	0.264	**-0.42**	<0.001	0.22	0.005	0.07	0.362
Min. Temp. Cold. Month	**-0.54**	<0.001	0.22	0.006	-0.07	0.370	-0.12	0.140
Annual Precipitation	**0.73**	<0.001	**0.33**	<0.001	0.13	0.110	**0.33**	<0.001
Precip. Sean. CV	**0.27**	<0.001	**0.49**	<0.001	-0.02	0.845	-0.09	0.235

(continues)

(continued)

Space								
Latitude	**-0.90**	<0.001	-0.18	0.023	-0.18	0.021	-0.17	0.035
Longitude	0.17	0.030	**-0.72**	<0.001	**0.26**	<0.001	**0.30**	<0.001
Lat.*Long.	-0.01	0.878	**-0.78**	<0.001	0.19	0.014	**0.27**	<0.001
Lat.*Lat.	**-0.90**	<0.001	-0.18	0.023	-0.18	0.021	-0.17	0.035
Long.*Long.	0.17	0.030	**-0.72**	<0.001	**0.26**	<0.001	**0.30**	<0.001
Lat.*Lat.*Long.	-0.15	0.055	**-0.78**	<0.001	0.11	0.161	0.17	0.033
Lat.*Long.*Long.	0.05	0.554	**-0.75**	<0.001	0.21	0.007	**0.32**	<0.001
Lat.*Lat.*Lat	**-0.90**	<0.001	-0.18	0.023	-0.18	0.021	-0.17	0.035
Long.*Long.*Long.	0.17	0.030	**-0.72**	<0.001	**0.26**	<0.001	**0.30**	<0.001

Nominal (P values)	DCA1	DCA2	DCA3	DCA4
Environment				
Stream	0.467	0.643	0.045	0.327
Timber	0.257	0.460	0.360	0.931
Bare	**0.006**	0.079	**<0.001**	0.090

TABLE 3. DCA of 161 sites based on small species. Spearman's correlation coefficients between explanatory variables and sites scores on the first four axes for continuous and ordinal variables. Values of Spearman correlation coefficient (r_S) and significance probabilities (P) are shown. Significant correlations after Bonferroni correction are in bold (the cut level was P = 0.0014). Whitney U test was used for testing relationships between nominal variables and sites scores on the DCA axes. Values of P< 0.05 in bold. Eigenvalues: 1st axis 0.218, 2nd axis 0.085, 3rd axis 0.060, 4th axis 0.049. Percentage variance of the species data explained: 1st axis 22.2%, 2nd axis 8.5%, 3rd axis 6.8%, 4th axis 5.0%.

	DCA1		DCA2		DCA3		DCA4	
Continuous and ordinal	rs	P	rs	P	rs	P	rs	P
Environment								
Rock	**0.44**	<0.001	-0.01	0.921	**0.31**	<0.001	-0.01	0.875
Limestone	**0.40**	<0.001	-0.08	0.339	0.10	0.215	0.17	0.028
Slope	**0.51**	<0.001	0.10	0.223	**0.46**	<0.001	-0.05	0.542
Wet	**-0.30**	<0.001	0.06	0.465	-0.20	0.011	0.07	0.362
Disturbance	-0.02	0.764	0.08	0.296	0.06	0.469	0.02	0.756
Conifers	**-0.39**	<0.001	-0.11	0.163	-0.18	0.022	-0.11	0.181
Bee	**0.50**	<0.001	0.08	0.311	0.18	0.022	0.01	0.937
mdw	0.05	0.532	-0.24	0.003	-0.04	0.601	0.01	0.861
aldwil	**-0.29**	<0.001	0.03	0.679	-0.21	0.007	0.10	0.216
Ash	-0.01	0.910	0.05	0.541	-0.13	0.101	0.21	0.007
weth	-0.12	0.117	**0.32**	<0.001	0.04	0.641	0.14	0.075
dogsm	0.16	0.044	**-0.39**	<0.001	-0.08	0.293	0.01	0.876
richh	0.15	0.059	**-0.30**	<0.001	-0.16	0.048	0.04	0.615
acidh	-0.17	0.030	0.05	0.549	0.05	0.517	-0.11	0.180
Altitude	**0.45**	<0.001	**0.47**	<0.001	**0.41**	<0.001	-0.12	0.137

(continues)

(continued)

Wet combination	**-0.26**	<0.001	0.11	0.176	-0.20	0.011	0.13	0.090
Rich combination	**0.31**	<0.001	**-0.29**	<0.001	-0.04	0.604	0.08	0.288
Poor combination	0.14	0.087	0.03	0.665	0.05	0.538	-0.08	0.332
Climate								
Mean Annual Temp.	0.01	0.882	-0.11	0.160	-0.01	0.888	0.20	0.010
Max. Temp. Warm. Month	-0.13	0.089	0.05	0.539	0.03	0.707	0.19	0.017
Min. Temp. Cold. Month	-0.20	0.010	**-0.46**	<0.001	**-0.28**	<0.001	0.11	0.147
Annual Precipitation	**0.56**	<0.001	**0.48**	<0.001	**0.46**	<0.001	-0.10	0.205
Precip. Sean. CV	**0.28**	<0.001	0.09	0.275	0.19	0.016	0.01	0.914
Space								
Latitude	**-0.69**	<0.001	**-0.53**	<0.001	**-0.41**	<0.001	-0.05	0.530
Longitude	-0.12	0.125	0.08	0.298	-0.02	0.759	0.12	0.143
Lat.*Long.	**-0.29**	<0.001	0.01	0.883	-0.07	0.360	0.08	0.318
Lat.*Lat.	**-0.69**	<0.001	**-0.53**	<0.001	**-0.41**	<0.001	-0.05	0.530
Long.*Long.	-0.12	0.125	0.08	0.298	-0.02	0.759	0.12	0.143
Lat.*Lat.*Long.	**-0.34**	<0.001	-0.09	0.241	-0.17	0.034	0.07	0.378
Lat.*Long.*Long.	-0.24	0.003	0.03	0.723	-0.05	0.506	0.11	0.177
Lat.*Lat.*Lat	**-0.69**	<0.001	**-0.53**	<0.001	**-0.41**	<0.001	-0.05	0.530
Long.*Long.*Long.	-0.12	0.125	0.08	0.298	-0.02	0.759	0.12	0.143

Nominal (P values)	DCA1	DCA2	DCA3	DCA4
Environment				
Stream	0.067	**0.035**	0.665	0.814
Timber	0.767	0.962	0.147	0.569
Bare	**0.029**	**0.049**	**0.019**	0.063

TABLE 4. Results of partitioning variation using partial CCAs and Monte Carlo permutation test with 1999 runs. In each group only the five most powerful predictors were chosen (based on separated CCAs, forward selection and MC test for each group of variables). All were significant - MC test. The right section shows the independent variation for each group, i.e. variation explained when other two groups of variables were used as covariables. AnnPrec, annual precipitation; PrecSeas, coefficient of variation in seasonal precipitation, MaxTemWa, maximum temperature in warmest month, MeanAnnTemp, mean annual temperature; MinTempCo, minimum temperature in coldest month.

	All variation explained		Independent variation	
Geography	Order in Forward selection	%	Order in Forward selection	%
Latitude	1	9.9	1	3.0
Longitude	2	6.1	3	3.5
Lat*Long	3	5.6	4	2.2
Lat*Lat	4	3.4	2	1.7
Long*Long*Long	5	2.7	5	0.9
total		**27.2**		**11.3**

	All variation explained		Independent variation	
Climate	Order in Forward selection	%	Order in Forward selection	%
AnnPrec	1	7.9	3	1.8
PrecSeas	2	4.0	1	1.4
MaxTemWa	3	3.9	4	0.9
MeanAnnTemp	4	3.3	5	0.9
MinTempCo	5	2.0	2	1.1
total		**21.2**		**6.0**

	All variation explained		Independent variation	
Environment	Order in Forward selection	%	Order in Forward selection	%
Altitude	1	6.8	2	1.7
Rock	2	4.6	1	1.3
Beech	3	2.0	4	1.3
Lime	4	1.8	3	0.7
Dogs Mercury	5	1.8	5	0.6
total		**17.1**		**5.6**

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FEEDING PREFERENCES OF TWO PREDATORY SNAILS INTRODUCED TO HAWAII AND THEIR CONSERVATION IMPLICATIONS

Wallace M. Meyer III[1, 2]* & Robert H. Cowie[1]

ABSTRACT

Two introduced predatory land snails, *Euglandina rosea* and *Oxychilus alliarius*, have been implicated in the decline of native Hawaiian and Pacific island land snails. We examined the feeding ecology of *E. rosea* and *O. alliarius*, focusing first on prey size and species preferences, and second on quantifying consumption rates, which is necessary to address the possible levels of impact on natural populations. In prey-size preference experiments, *E. rosea* always consumed more snails in the smaller size class. In prey-species preference experiments, *E. rosea* preferred snails to slugs but showed no preference among snail species. Size preference experiments indicated that *O. alliarius* will only consume prey less than 3 mm in shell length. In prey species preference experiments *O. alliarius* consumed every snail species offered but did not consume either of the slug species. Consumption rates were positively related to the size of the predator, with the larger predator, *E. rosea*, having a much higher consumption rate than *O. alliarius*, and with larger *E. rosea* consuming more than smaller *E. rosea*. These results have unfortunate conservation ramifications, because since they indicate that both *E. rosea* and *O. alliarius* are generalist snail predators feeding preferentially on the smallest snails, although they rarely eat slugs. Since many extant Hawaiian and Pacific island snail species are small, they are at risk of predation by these two introduced predators, while invasive slugs – there are no native slugs in Hawaii – may not be impacted by them.

Key words: consumption rate, *Euglandina rosea*, Gastropoda, *Oxychilus alliarius*, slug.

INTRODUCTION

Land-snail faunas world-wide are under extreme threat, and have the dubious honor of having the highest number of documented extinctions of any major taxonomic group (Cowie, 2004; Nash, 2004; Lydeard et al., 2004). The 2008 *IUCN Red List of Threatened Species* (IUCN, 2008) included 1,994 gastropods (snails and slugs), of which 271 were listed as extinct. Native Pacific island land snails, in particular, are recognized for their diversity, high levels of endemism and the fact that they are under extreme threat, with perhaps as many as 50% of the species having disappeared (Lydeard et al., 2004). For example, extinction of the tree-snail species (Achatinellinae in Hawaii and Partulidae on other Pacific islands) has been catastrophic (Clarke et al., 1984; Hadfield, 1986; Murray et al., 1988; Cowie, 1992; Hadfield et al., 1993; Coote & Loève, 2003).

In Hawaii, the situation may be especially critical. The native Hawaiian land-snail fauna used to be extremely diverse (over 750 species) and exhibited extremely high endemism (over 99%) (Cowie et al., 1995), but estimates of extinction of these unique species range from 65–75% (Solem, 1990) to 90% (Cowie, 2002; Lydeard et al., 2004). For example, the Amastridae, an endemic Hawaiian family of more than 300 species (Cowie et al., 1995), may now be reduced to as few as ten or so species existing in tiny, highly localized, remnant populations. The Endodontidae in Hawaii and throughout the Pacific appear reduced to sparse populations on those islands they formerly inhabited (Lydeard et al., 2004; Meyer, 2006).

Two introduced predatory land snails, *Euglandina rosea* and *Oxychilus alliarius*, have been implicated in the decline of the native land-snail faunas. *Euglandina rosea* was purposely introduced to Hawaii in 1955 to control populations

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[1]Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawaii at Manoa, 3050 Maile Way, Gilmore 408, Honolulu, Hawaii 96822, U.S.A.
[2]Department of Zoology, University of Hawaii at Manoa, 2538 McCarthy Mall, Edmonson 152, Honolulu, Hawaii 96822, U.S.A.
*Corresponding author: meyerwal@hawaii.edu

of another introduced snail, *Achatina fulica*, the giant African snail (Davis & Butler, 1964; Simberloff, 1995; Civeyrel & Simberloff, 1996; Cowie, 2001). Subsequently, it was introduced elsewhere (Mead, 1961) and is now widely distributed throughout the Pacific (Cowie, 2001; Cowie et al., 2008). Unfortunately, the introduction of *E. rosea* has not reduced *A. fulica* populations (Civeyrel & Simberloff, 1996; Cowie, 2001), but has been associated with the decline of many of the tree-snail species, not only in Hawaii but elsewhere in the Pacific (Clarke et al., 1984; Hadfield, 1986; Hadfield et al., 1993; Murray, 1993; Gerlach, 2001; Coote & Loève, 2003), and may have caused the extinction of other native land-snail species (Cowie, 1998, 2001). Native to Europe, *O. alliarius* was first recorded in the Hawaiian Islands in 1937 (Cowie, 1997). Ignored until more recently, *O. alliarius* has become extremely abundant on the islands of Maui (Severns, 1984) and Hawaii (Meyer & Cowie, in press) and now occurs also on Molokai and Kauai (Cowie, 1997) and on Oahu (W. M. M., personal observation). *Oxychilus alliarius* is omnivorous, but is known as a snail predator; it may have played a role in the decline of the native Hawaiian land snail fauna, as it is thought to have done in New Zealand (Barker & Efford, 2004), and it could be a threat to the remaining extant species (Severns, 1984).

Certain aspects of the feeding ecology of *E. rosea* have been studied, including feeding behavior and prey trail following (Cook, 1985a, b; Gerlach, 1999), prey-size preferences (Chiu & Chou, 1962; Davis & Butler, 1964; Nishida & Napompeth, 1975; Cook, 1989a, b; Gerlach, 1999), and prey-species selection in the wild (Griffiths et al., 1993). However, extrapolating from these results to determine the effect *E. rosea* is having on land snail faunas as a whole is difficult, especially because consumption rates and the reasons for prey preferences have not been adequately addressed. For instance, experiments that addressed species preferences did not control for prey size among prey species offered (Cook, 1989a, b). Instead, total weight of each species was standardized. However, if prey size is as important as suggested (Chiu & Chou, 1962; Davis & Butler, 1964; Nishida & Napompeth, 1975; Cook, 1989a, b), the fact that the smaller species were preferred in choice experiments tells us little about preferences for specific species. Also, the microcosms used in these experiments (clear glass dishes) did not provide conditions permitting all possible predator avoidance behaviors. We know of no reports of feeding preference experiments on *O. alliarius*.

Despite the two predators' reputations, relatively little attention has been paid to the biology of either. Our objective was to examine the feeding ecology of *E. rosea* and *O. alliarius*, focusing first on prey-size and species preferences, and second on quantifying consumption rates in order to address the possible levels of impact on natural populations.

METHODS

All experiments were conducted with snails collected from the wild less than one month previously. They were allowed to acclimate to laboratory conditions for at least three days prior to any experiments. Both predator species were fed a diet of *Bradybaena similaris*, a common invasive snail in Hawaii. *Bradybaena similaris* was not used as a prey species in the subsequent experiments. All animals were maintained in the same kind of containers as those used in the experiments: 30 x 20 x 30 cm for *E. rosea*; 5 x 5 x 5 cm for *O. alliarius*. Laboratory temperatures were 19–24°C during the experiments.

Different methods were used to assess the feeding behavior of each predator, since *E. rosea* has a much higher consumption rate than *O. alliarius*. The primary difference between the methods were that experiments examining *E. rosea* preferences (prey size and species) were choice experiments, with individual predators given a choice between two prey options, while *O. alliarius* experiments were no-choice, as each predator was only offered one prey option. A different individual was used as a predator for every replicate across all experiments. For the *E. rosea* experiments, because hundreds of prey individuals were required, we used only non-native species, from five distinct families: two slug species (*Veronicella cubensis*, *Deroceras laeve*) and three snail species (*Achatina fulica*, *Paropeas achatinaceum*, *Succinea tenella*). These species are phylogenetically diverse, such that the results should be generalisable. Inclusion of a species of non-native Succineidae, a family that has proportionately more extant native species in Hawaii than any other family (Rundell et al., 2004), also provides more specific relevance to the local fauna. Given the much lower consumption rates of *O. alliarius*, it was reasonable to include native species as prey (*Succinea* spp., *Philonesia* sp., *Tornatellides* spp.) in addition to common invasive species (*Arion intermedius*, *Deroceras laeve*, *Paropeas achatinaceum*), all of which occur in areas in Hawaii with abundant *O. alliarius*.

Prey-Size Preference

Euglandina rosea

Seventeen adult *E. rosea* (35–55 mm shell length) were starved for two days prior to experimentation to standardize hunger levels. Each experimental container was filled with 2 cm of soil, a calcium source (cuttle bone), and some lettuce leaves as food for the prey. The prey (*A. fulica*) were divided into four size classes (0–1, 1–2, 2–3, and 3–4 cm shell length). Ten snails from two of the four size classes were offered to one *E. rosea* after the prey had been allowed to acclimate to the container for one day. *Euglandina rosea* consumes small snails whole as well as eating larger snails via the shell aperture. Every 12 h consumed individuals were replaced with new individuals of the same size class. No more than four snails of one size class were replaced at one interval, so a choice of prey was always available. Experiments were run for four to ten days depending on consumption rates and prey availability. At the end of the experiments, the numbers of small prey and large prey consumed were added up for each container. Differences in preference between small and large prey were assessed by comparing the number of containers with more small prey consumed to containers with more large prey consumed using a sign test (Sokal & Rohlf, 1995).

Oxychilus alliarius

Adult *O. alliarius* (6–10 mm shell width) were offered prey (native *Succinea* spp. from areas where *O. alliarius* is abundant) of various sizes. One prey item was placed in a container with 2 cm of soil and one leaf of *Broussaisia arguta* to provide it with habitat and food. Prey were divided into six size classes (1–3, 3–5, 5–7, 7–9, 9–11, and 11–13 mm shell length). Seventy-four experimental replicates each contained one adult *O. alliarius* in addition to the prey snail, while 48 control replicates each contained only the prey snail. Each trial was run for seven days at the end of which prey were recorded as alive or dead. We have only seen *O. alliarius* feed via the aperture, as opposed to consuming the entire prey snail including its shell. Differences in mortality between control and experimental treatments for each size class were tested using the log odds ratio, *l(o)* (Sokal & Rohlf, 1995), as follows:

$$l(o) = \ln\left[\frac{((N_{11} + 0.5) / (N_{12} + 0.5))}{((N_{21} + 0.5) / (N_{22} + 0.5))}\right]$$

which is useful because it permits standard errors, $s_{l(o)}$, to be calculated from binary data, as follows:

$$s_{l(o)} = \left(\frac{1}{(N_{11} + 0.5)} + \frac{1}{(N_{21} + 0.5)} + \frac{1}{(N_{12} + 0.5)} + \frac{1}{(N_{22} + 0.5)}\right)^{1/2}$$

where N_{11} is the number of experimental replicates in which the prey survived; N_{12} is the number of control replicates in which the species survived; N_{21} is the number of experimental replicates in which the prey died; N_{22} is the number of control replicates in which the prey died.

If mortality is equal in control and experimental treatments the log odds ratio is zero. Significant differences in mortality were determined when a log odds ratio was: 1) further than 1.96 standard errors away from zero ($p < 0.05$), and 2) further than 2.576 standard errors away from zero ($p < 0.01$).

Prey-Species Preference

Euglandina rosea

Prey-species preference of *E. rosea* was assessed by offering a choice between two of the five prey species listed above. Prey were 5–15 mm in extended foot length. The size distribution, that is, the numbers of snails/slugs of various sizes in each trial, was matched between the two prey species in order to minimize any effect of size in determining species preference. Using the length of the extended foot, rather than a shell size measure, was necessary since some of the prey choices were slugs (*V. cubensis* and *D. laeve*). Of the ten possible pair-wise combinations, eight were tested (*D. laeve* vs. *S. tenella* and *D. laeve* vs. *A. fulica* were excluded because of lack of adequate numbers of *D. laeve*). There were ten replicates for each pair-wise combination. A different *E. rosea* and 20 prey (ten individuals of two species) were used in each replicate. The number of each type of prey consumed (no replacement) over a 24 h period was recorded.

A preference index was calculated for each predator in each replicate/container using a version of Manly's α index that accounts for variable prey populations, that is where prey are not replaced (Krebs, 1999), as follows:

$$\alpha_i = \frac{\text{Log } p_i}{\sum_{i=1}^{m} p_j}$$

where α_i is Manly's α (preference index) for prey type i; p_i, and p_j are the proportions of prey i or j remaining at the end of the experiment (i = 1, 2, 3, … m; j = 1, 2, 3, … m), that is, e_i/n_i in which e_i is the number of prey type i remaining uneaten at the end of the experiment, n_i is the initial number of prey type i in the experiment, and m is the number of prey types (similarly for e_j/n_j). Values for this index range between 0 and 1; values > 0.5 indicate preference and values < 0.5 avoidance. Significance of divergence from 0.5 (no preference) was assessed with single sample t-tests.

Oxychilus alliarius

Prey-species preference of *O. alliarius* was assessed by offering adults (6–10 mm maximum shell width) a single prey individual of various species (< 3 mm maximum shell dimension for snails and similarly sized slugs determined by matching foot size between slugs and snails) following the same procedure and analyses as in the *O. alliarius* prey-size experiments. Prey species used were: the native snail species *Succinea* spp. (33 experimental trials, 18 controls), *Tornatellides* spp. (13,15), and *Philonesia* sp. (12,12); the invasive snail species *P. achatinaceum* (10,8); and the invasive slug species *A. intermedius* (17,17) and *D. leave* (11,10).

Consumption Rate

Euglandina rosea

Twelve *E. rosea* were used in consumption rate experiments (seven adults 40–55 mm shell length, five juveniles 10–25 mm shell length). For a week prior to the experiments, they were provided with prey snails (*A.fulica*, *P. achatinaceum*, *B. similaris*) of various sizes *ad libitum*. Snails were not starved, so that the consumption rate measured would be a more natural rather than a maximum rate. Thirty prey snails (non-native *Succinea tenella*, 15 in the size range 1–5 mm and 15 in the range 5–12 mm shell length) were then offered to an individual *E. rosea* for a period of 24 h. Each prey snail was weighed to the nearest 0.0001 g and individually marked. At the end of the experiment, the number of snails remaining alive was counted, and any shells remaining from consumed snails were weighed to the nearest 0.0001 g, these weights being subtracted from the weights of those snails at the beginning of the experiment to give a value of the weight consumed. Adding this amount to the known weight of snails consumed whole provided a value for the total weight consumed. Growth of prey snails over the 24 h of the experiment was assumed to be negligible. Wet weight consumed was regressed on *E. rosea* size. Manly's α index (described above) was used to define preference, followed by a Mann-Whitney U-test to test for significant differences in preference for small prey (1–5 mm) vs. large prey (5–12 mm) between adult and juvenile *E. rosea*.

TABLE 1. Number of *Achatina fulica* eaten by *Euglandina rosea* when given a choice between two size classes. Each experiment used a different *E. rosea* individual.

		Number Eaten	
Size Classes	Duration (days)	Smaller	Larger
0–1 vs. 1–2 cm	4	10	2
	4	9	0
	4	13	0
	4	2	0
0–1 vs. 2–3 cm	5	11	0
	5	4	0
0–1 vs. 3–4 cm	5	10	0
1–2 vs. 2–3 cm	5	4	0
	5	4	0
	5	3	0
	5	0	0
1–2 vs. 3–4 cm	4	3	0
	4	1	0
2–3 vs. 3–4 cm	10	0	0
	10	2	0
	10	1	0
	10	0	0
Total		77	2

TABLE 2. Predation of *Oxychilus alliarius* on native *Succinea* spp. of different size classes (shell length). P-Value based on log odds ratio tests. NS indicates P-Values > 0.05.

	Control		Experimental		
Size Class	No. Survived	No. Died	No. Survived	No. Died	P-value
0–3 mm	20	0	3	33	< 0.01
3–6 mm	16	0	16	0	NS
6–9 mm	17	0	17	0	NS
> 9 mm	12	1	12	0	NS

Oxychilus alliarius

Twelve *O. alliarius* were used in consumption-rate experiments. They were collected from the field and placed directly into individual containers each with five prey snails (*Succinea* spp., 1–3 mm shell length) for seven days. Each prey item was weighed to the nearest 0.0001 g and was individually marked prior to being placed in the container. At the end of the experiment, the snails remaining alive were counted and any shells remaining from consumed snails were weighed to the nearest 0.0001 g, these weights being subtracted from the weights of those snails at the beginning of the experiment to give a value of the weight consumed.

RESULTS

Prey-Size Preference

Regardless of which snail combination *E. rosea* was offered (Table 1), in all cases in which at least one prey was consumed (14 of 17) more of the smaller snails were consumed (sign test: $P < 0.001$). Even being extremely conservative and considering the three instances in which no prey were eaten and the one in which two of the larger prey were eaten (in the 0–1 vs. 1–2 cm trials) as supporting the null hypothesis of no preference, gives a two-sided P-Value of 0.049. Pooling the data, 77 snails in the smaller size class were consumed

TABLE 3. Results of pair-wise comparison of preferences (Manly's α) of *E. rosea* among five prey species: *Vc = Veronicella cubensis*, *Dl = Deroceras laeve* (the two slug species), *Pa = Paropeas achatinaceum*, *Af = Achatina fulica*, *St = Succinea tenella*. Values of α > 0.5 indicate preference; Values < 0.5 indicate avoidance. A single sample t-test was used to test if the preference indices differed significantly from 0.5. Statistically significant differences are indicated by asterisks (P-values are < 0.01 in all cases).

Treatment	Manly's α Preference Index (SE)	
Snails vs. Snails		
Pa vs. *St*	*Pa* = 0.417 (0.221)	*S* = 0.583 (0.211)
Pa vs. *Af*	*Pa* = 0.482 (0.255)	*Af* = 0.518 (0.255)
Af vs. *St*	*Af* = 0.459 (0.151)	*S* = 0.541 (0.151)
Slugs vs. Snails		
Vc vs. *Pa*	*Vc* = 0.039 (0.089)*	*Pa* = 0.961 (0.089)*
Vc vs. *St*	*Vc* = 0.009 (0.024)*	*S* = 0.991 (0.024)*
Vc vs. *Af*	*Vc* = 0.029 (0.086)*	*Af* = 0.971 (0.086)*
Dl vs. *Pa*	*Dl* = 0.137 (0.082)*	*Pa* = 0.863 (0.082)*
Slugs vs. Slugs		
Dl vs. *Vc*	*Dl* = 0.893 (0.168)*	*Vc* = 0.106 (0.168)*

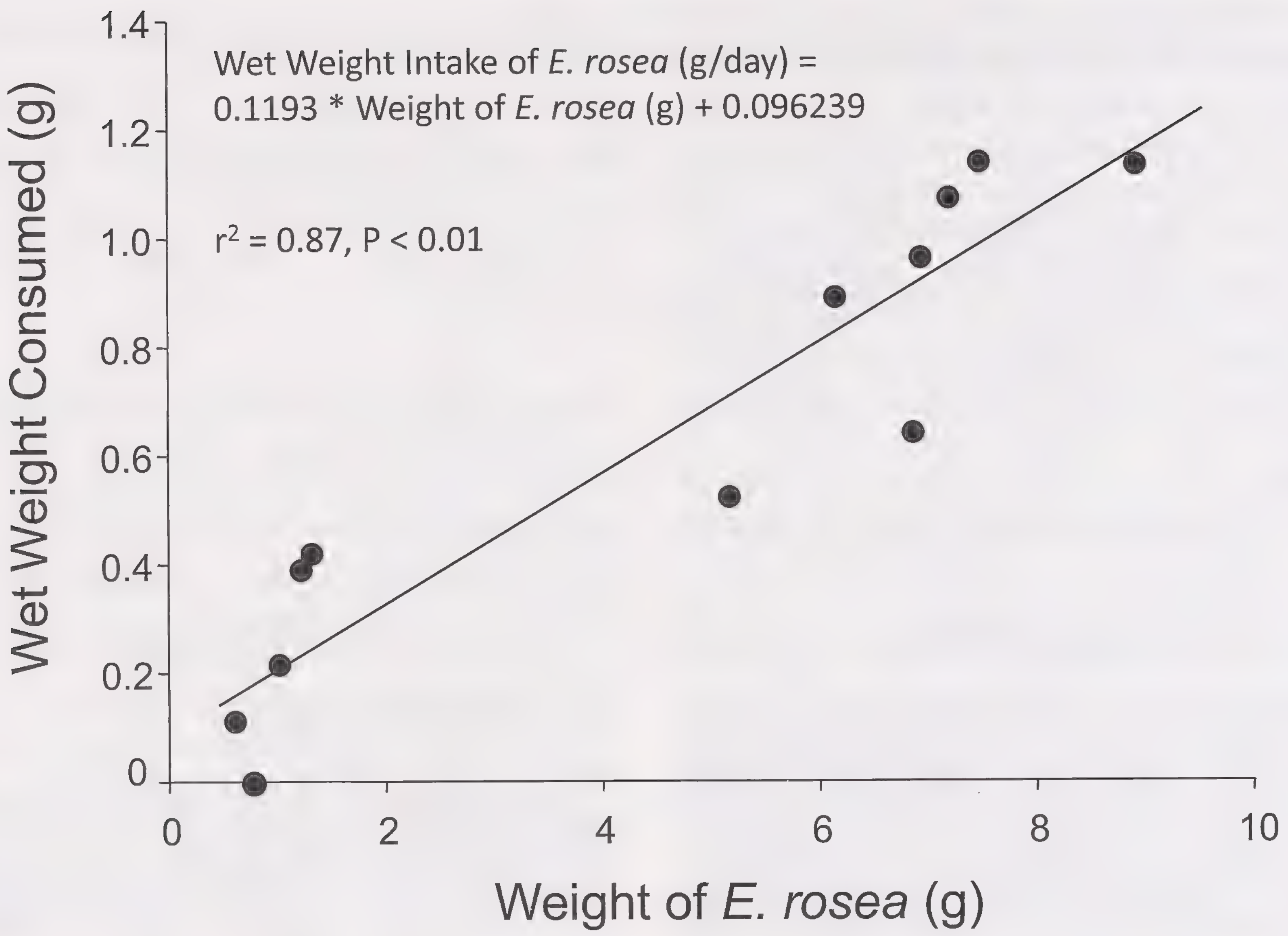

FIG. 1. Consumption rate (wet weight consumed during a 24 h period) vs. weight of *Euglandina rosea*.

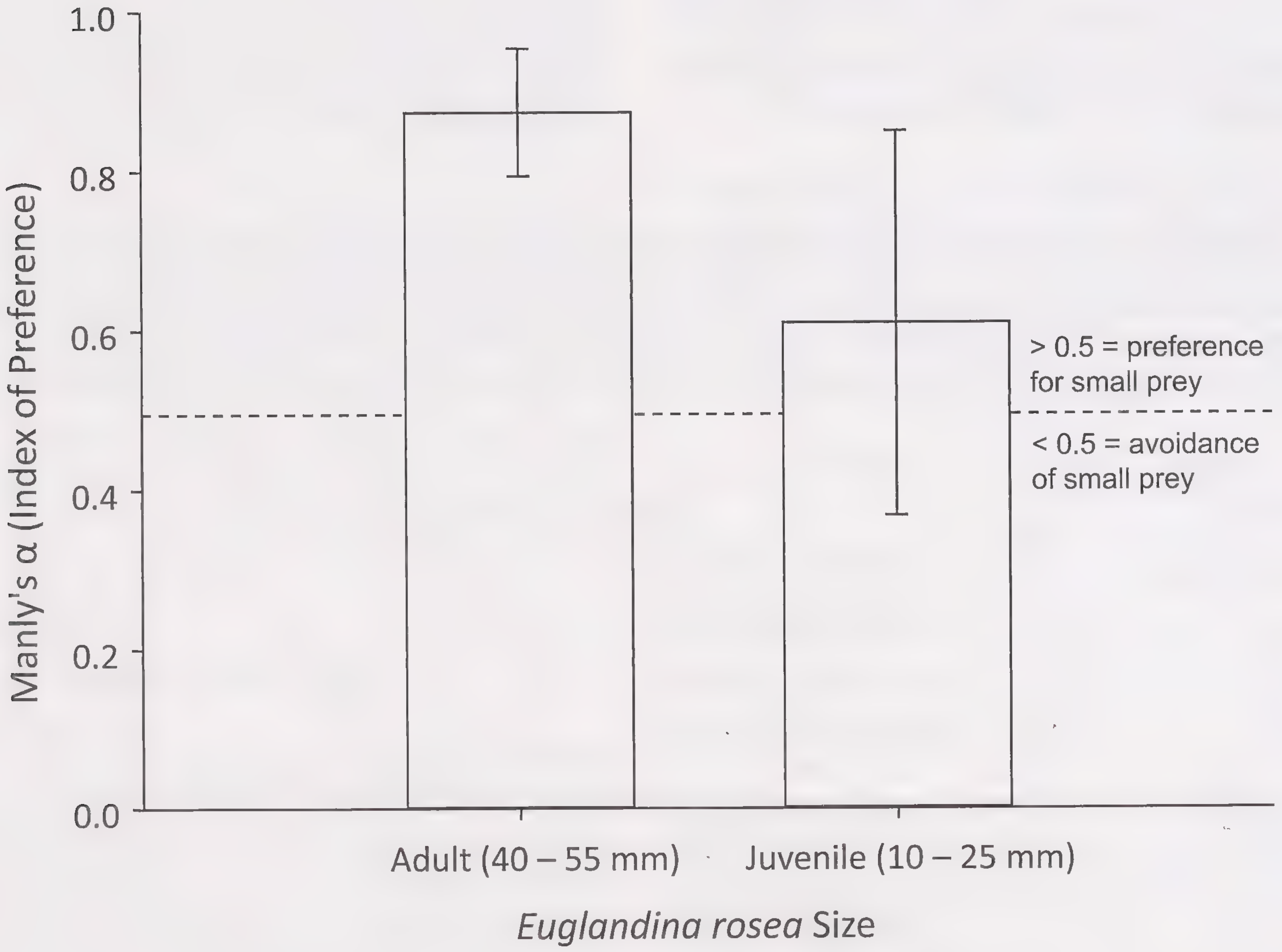

FIG. 2. Manly's α indices for adult and juvenile *E. rosea* preference for smaller prey (1–5 mm shell length) or larger prey (5–12 mm shell length). Error bars represent ± 1 standard error.

TABLE 4. Predation of *Oxychilus alliarius* on native and introduced snails (*Succinea* spp., *Tornatellides* spp., *Philonesia* sp., *P. achatinaceum*) and slugs (*D. laeve* and *A. intermedius*). P-Value based on log odds ratio tests. NS indicates P-values > 0.05. *Native species.

	Control		Experimental		
Taxa	No. Survived	No. Died	No. Survived	No. Died	P-value
Snails					
Succinea spp.*	18	0	6	25	< 0.01
Tornatellides spp.*	14	1	1	12	< 0.01
Philonesia sp.*	12	0	4	8	< 0.05
P. achatinaceum	8	0	1	9	< 0.05
Slugs					
D. laeve	10	0	10	1	NS
A. intermedius	16	1	17	0	NS

compared to only two from the larger size class. *Euglandina rosea* thus prefers smaller snails when given a choice.

In the *O. alliarius* experiments, no snail above 3 mm in shell length was preyed upon compared to 33 of the 36 (91%) that were 3 mm in shell length or less; hence, there was no significant difference between control and experimental treatments except in the 0–3 mm prey size category (Table 2).

Prey-Species Preference

Euglandina rosea always preferred the snail over the slug species but showed no preference among the three snail species (Table 3). *Veronicella cubensis* was the least preferred prey, and *D. laeve* seemed to be preferred over *V. cubensis* but was less attractive than *P. achatinaceum* and therefore probably also less attractive than the other snail species (*S. tenella* and *A. fulica*).

Oxychilus alliarius readily consumed all snail species offered, as indicated by the much higher mortality in the experimental treatments, but there was no difference in mortality between control and experimental treatments for the two slug species (Table 4).

Consumption Rate

Wet weight consumed increased as *E. rosea* weight increased (Fig. 1). The high r^2 value (Fig. 1) suggests that this mathematical model describes the relationship well. The regression line was not forced to go through the origin but the y-intercept was nevertheless close to it. Adult *E. rosea* preferred smaller snails to larger snails ($\alpha = 0.874$, SE = 0.080; T =12.35, df = 6, $P < 0.001$), but juvenile *E. rosea* showed no preference ($\alpha = 0.609$, SE = 0.241; T = 0.905, df = 3, P = 0.432). The difference in preference was significant (U = 26.0, P = 0.023; Fig. 2).

The consumption rate for *O. alliarius* was 0.00097 (± 0.00019 SE) g of wet weight per day, which equates to about one snail (1–3 mm maximum shell dimension) consumed by each individual during the experiment (7 days).

DISCUSSION

Small snails were more vulnerable to predation by both *E. rosea* and *O. alliarius*. While *O. alliarius* can only consume small snails (less than 3 mm maximum shell dimension), *E. rosea* can consume larger prey. However, when given a choice of prey sizes, *E. rosea* will eat significantly more small snails than large snails (Table 1, Fig. 2). These results are consistent with other studies of prey size preference in *E. rosea* (Chiu & Chou, 1962; Davis & Butler, 1964; Nishida & Napompeth, 1975; Cook, 1989a, b; Griffiths et al., 1993; Gerlach, 1999). However, our results indicate that this size preference is relative not absolute, that is, mid-sized prey (1–2 or 2–3 cm in shell length) are not eaten if presented along with smaller prey, but are eaten if presented with larger prey (Table 1). It is not simply that larger prey cannot be eaten. This behavioral flexibility is interesting, and perhaps important for the dynamics of the interaction, although the data are too few to evaluate further. Nonetheless, our results and those of other studies indicate that differences in size among prey can influence the predatory behavior of *E. rosea*, and thus confound conclusions of prey species preference if prey size is not appropriately controlled in experiments.

The size of *E. rosea* is also important in understanding prey-size preference. Although counter-intuitive, larger *E. rosea* had a stronger preference for smaller prey (Fig. 2). This may be because large *E. rosea* have a greater ability to consume snails whole. Thus, the impact of large *E. rosea* on small snails may be even greater. In the field, a large majority of the species consumed by *E. rosea* were swallowed whole (Griffiths et al., 1993). One reason usually proposed for *E. rosea*'s preference for smaller snails is calcium intake. Cook (1989a) suggested that there may be a compromise between the input of important nutrients, primarily calcium, when the prey is consumed whole (shell included), and the caloric intake that comes from the consumption of just body tissue.

Both predators exhibited a clear preference for the snail species over the slug species in all tests (Tables 3, 4). In all experiments examining *E. rosea*'s preference between snails and slugs, it preferred the snails, while showing no preference among snail species in snail vs. snail experiments (Table 3). However, between the slugs, *E. rosea* did prefer *D. laeve* over *V. cubensis* (Table 3). *Oxychillus alliarius* showed similar preferences, consuming all snail species offered but no slugs (Table 4). There are no native slugs in Hawaii, but non-native slugs are abundant and widespread (Cowie et al., 2008; Meyer & Cowie, in press) and negatively influence Hawaiian ecosystems by reducing survivorship of young native plants (Joe & Daehler, 2008). Thus, predation on slugs could *a priori* be seen as a positive counter balance to the introduction of these predatory snails, but unfortunately, our results show that both *O. alliarius* and *E. rosea* prefer snails to slugs, and many of the slugs reach sizes well above the preferred size range of both *O. alliarius* and *E. rosea*.

Consumption rate was related to predator size. That of *O. alliarus* was much lower than that of *E. rosea*, reflecting the much smaller size of *O. alliarius*; and within *E. rosea* consumption rate increased with predator size (Fig. 1). However, the impact of *O. alliarius* on native snails may still be considerable because of the high densities this species can reach, notably at higher elevations on the islands of Maui and Hawaii.

Many of the extant native Pacific Island snails are small (e.g., numerous endodontids, helicinids, vertiginids, and achatinellids (excluding Achatinellinae) are < 3 mm in maximum shell dimension for their entire life) compared to some of the abundant introduced snails/slugs that have become established (e.g., *Achatina fulica*, *Cornu aspersum*, *Limax maximus*, *Veronicella cubensis*, and *Meghimatium striatum*) (Cowie et al., 2008; Joe & Daehler, 2008; Meyer & Cowie, in press). Most survive only in sparse populations (Lydeard et al., 2004; Meyer, 2006). The results indicate that *O. alliarius* and *E. rosea* would readily consume these native snails. Some native succineid species are also relatively small during the early stages of their life history, being less than 1 mm in shell length at hatching and rarely exceeding 13 mm in shell length (Brown et al., 2003; Rundell & Cowie, 2003; W. M. M., personal observations), making them vulnerable to *O. alliarius* for the first few months of their lives and to *E. rosea* for their entire lives. Achatinellids are more diverse in size. For instance, many Achatinellidae (with the exception of Achatinellinae) are widely but patchily distributed and extremely small (many < 3 mm in shell length) and are thus vulnerable to predation by both predators. Conversely, Achatinellinae, the Hawaiian tree snails, are larger. For instance, *Achatinella mustelina* are > 4 mm at birth and reach sizes greater than 21 mm in shell length (Hadfield & Mountain, 1980; Hadfield et al., 1993). Thus, while they are not likely to be consumed by *O. alliarius*, their populations have declined following the introduction of *E. rosea* (Hadfield & Mountain, 1980; Hadfield, 1986; Hadfield et al., 1993), as have similar sized Partulidae (Pacific island tree snails) following the introduction of *E. rosea* elsewhere (Clarke et al., 1984; Murray et al., 1988; Murray, 1993; Gerlach, 2001; Coote & Loève, 2003).

Although consumption rates of *O. alliarius* are much lower than those of *E. rosea*, *O. alliarius* may be having a large impact on native land snails. Populations of *O. alliarius* are more dense than those of *E. rosea* in native rain forests, notably on the islands of Hawaii and Maui, and are becoming more prevalent on other islands at higher elevations where most of the extant native snails are found (Severns, 1984, Meyer & Cowie, in press). Endangered Hawaiian tree snails are probably not impacted by *O. alliarius*, as they are too large even at birth, but much of the remaining Hawaiian land snail diversity may well be affected. Nevertheless, to ascertain the real individual and combined impacts of these predators on the land snail community as a whole and on populations of species of particular interest will require data on the population densities and size distributions of these predators as well as on the life-histories of the prey species. Unfortunately, data on population densities and life-histories for both prey and predators are few. However, investigations of these aspects of their ecology, combined with the preference and consumption rate data presented here, would permit models to be developed describing the dynamics of this system, which could help biodiversity managers protect native populations and control alien species.

Although many Pacific land-snail species are extinct, many unique species remain, representing all of the native families. Knowledge of the feeding ecology of these predators is therefore essential if natural resources managers are to design better conservation strategies targeting these remaining native species. The results of this study are relevant beyond Hawaii as both predatory snails and the introduced species used as prey species in these experiments have been widely distributed by human activities (Solem, 1964; Cowie, 2002; Coote & Loève, 2003; Barker & Efford, 2004; Cádiz & Gallardo, 2007). This may be especially pertinent for understanding the threat of these predatory snail species in the Pacific, since many native Pacific island snail faunas are composed of species belonging to many of the same families (Cowie et al., 1995; Cowie, 1996). We still have the opportunity to protect these unique lineages from extinction, but this is dependent on monitoring the presence of these predators and evaluating their impacts.

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RESEARCH NOTES

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IDENTIFICATION OF POLYMORPHIC MICROSATELLITES FOR THE INTERMEDIATE HOST *ONCOMELANIA HUPENSIS* OF *SCHISTOSOMA JAPONICUM* IN CHINA

Shu H. Zhang[1], Qin P. Zhao[1, 2] , Ran Jiao[1] , Qian Gao[1] & Pin Nie[1*]

INTRODUCTION

The snail *Oncomelania hupensis* in the family Pomatiopsidae is the unique intermediate host of *Schistosoma japonicum* in some Far East countries. In China, this snail host is widely distributed, which determines largely the epidemic area of schistosomiasis. Despite the wide geographical distribution, the habitats of *O. hupensis* can be in general grouped into three ecological categories: (1) lake/marshland representing the shore area of lakes and the Yangtze River, (2) flood-plain and water channel representing the area in the flood-plain of the River and all water channel systems in the plain, and (3) hilly and mountainous regions, with the former in some areas in the middle and lower reaches of the Yangtze River, and the latter mostly in Sichuan, Yunnan provinces now, but also including Fujian, Guangdong provinces and Guangxi autonomous region (Zhou et al., 2005).

Snails distributed in these different habitats vary in their morphotypes. In general, two morphotypes, that is ribbed-shelled and smooth-shelled, have been reported in China (Liu et al., 1981; Davis et al., 1995). Snails distributed in areas of the middle and lower reaches of the Yangtze River, genetically defined as *O. hupensis hupensis,* are polymorphic with ribbed and smooth shells that always have a varix (Davis et al., 1995, 1999). Davis et al. (2006) recently demonstrated polymorphism in shell sculpture from heavily-ribbed shells to slightly-ribbed to smooth-shelled conditions in the same populations in canals in Hubei province. The ribbed shelled morph lives on the flood plains while smooth-shelled snail live in hilly areas or in canals removed from Yangtze River flooding (Davis et al., 1995, 1999, 2006). *Oncomelania hupensis robertsoni*, living in Yunnan and Sichuan provinces, is genetically quite distinct from *O. hupensis hupensis* with unique population genetic attributes (Wilke et al., 2006). *Oncomelania hupensis robertsoni* has a smooth shell, is uniformly smaller than *O. hupensis hupensis*, and never has a varix.

Oncomelania hupensis in Fujian province and in southwest China, Guangxi autonomous region, have smooth-shelled snails with pronounced varix (Liu et al., 1984; Davis et al., 1995). In Fujian province, genetic data support the classification *O. hupensis tangi*, while the classification *O. hupensis guangxiensis* in Guangxi autonomous region is still under consideration(Liu et al., 1981; Davis et al., 1995; Li et al., 2009). The subspecies of *O. hupensis* are significantly different from each other genetically. Overall, *O. hupensis* exhibits a high degree of genetic diversity throughout China. Several studies, using mitochondrial or nuclear genes or even allozymes have detected genetic difference in *O. hupensis* in China, and genetic diversity in *O. hupensis hupensis* and also in *O. hupensis robertsoni* (e.g., Davis et al., 1995, 1999; Wilke et al., 2000, 2006; Li et al., 2009; Zhao et al., 2010). In consideration of the wide geographical distribution, it is thus hypothesized, as also suggested by Davis et al. (1998, 2006), Wilke et al. (2006), and Zhao et al. (2010) that the intermediate host may have considerable genetic diversity at population level, thus within subspecies, which should be examined by using more powerful molecular markers such as microsatellite markers. The understanding of genetic diversity of the intermediate hosts may shed light on the genetic complexity of their parasite, *Schistosoma japonicum.*

Microsatellites or simple sequence repeats (SSRs) have been used widely and efficiently as molecular markers in studying population genetics and parentage and kinship analyses because of their high level of polymorphism and codominant Mendelian inheritance (O'Connell & Wright, 1997). In this study, we employed SSRs methods to screen polymorphic molecular markers from nuclear genome of *O. hupensis*, in order to provide effective microsatellite markers for investigating the population genetics of the intermediate hosts in China.

[1]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei Province, 430072, China
[2]Department of Parasitology, School of Basic Medical Science, Wuhan University, Wuhan, Hubei Province, 430071, China
*Corresponding author: pinnie@ihb.ac.cn

MATERIAL AND METHODS

Oncomelania hupensis were obtained with forceps from a wide range of schistosomiasis endemic areas in mainland China in six localities of five provinces, representing principally three different ecological categories (Table 1). The diagnosis of subspecies of *O. hupensis* followed that of Davis et al. (1995). Snails obtained were then brought back to laboratory, where they were washed and captured for several days, and those confirmed without parasite infection under a microscope were preserved in 95% ethanol. The head-foot muscle of about 200 snails from each locality were dissected out and fixed in 70% ethanol before being used for the extraction of the total genomic DNA using a standard proteinase K/SDS extraction method (Sambrook et al., 2001). The DNA concentration was determined by measuring the optical density using a BioPhotometer (Eppendorf, Germany). All samples were then diluted to a concentration of 50 ng/µl.

In order to obtain well-behaved polymorphic microsatellite sequences suitable for studying population genetics, it is necessary to mix the extracted total genomic DNA from samples of six localities (Table 1) for the construction of genomic library. Microsatellite-enriched genomic library for the repeat motif $(CA)_n$ and $(CT)_n$ was constructed essentially following the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (review in Zane et al., 2002). Enriched fragments were ligated into pMD18-T vector (TaKaRa) and propagated in the DH5α strain of *Escherichia coli*. After polymerase chain reaction (PCR) confirmation, 632 positive amplicons about 300–700 bp long were sequenced with ABI PRISM 3700.

The cloned sequences were analysed for the repeat regions using software Tandem Repeats Finder (Benson, 1999), and 174 putative microsatellite sequences were examined using primers designed with the software Primer3 (Rozen & Skaletsky, 2000). The polymorphism of these microsatellite sequences was further examined with *Oncomelania hupensis* collected from the above mentioned 6 localities (Table 1), and further analyzed with thirty snails collected from Jiangling County of Hubei Province, China in the present study. In general, the PCR amplification for detecting the polymorphism was carried out in a mixture of 25 µl volume on a PTC-100 thermocycler (Biorad, USA), containing 1 × PCR buffer (Tiangen), 50 ng genomic DNA, 0.25 µm for each primer, 150 µmol/L dNTPs, 1.5 mm $MgCl_2$ and 0.25 U Taq DNA polymerase (Tiangen), with the following programme: an initial denaturation at 94°C for 5 min; 35 cycles including denaturation at 94°C for 40 s, annealing at the proper temperature (Table 1) for 40 s and elongation at 72°C for 40 s, and a final elongation at 72°C for 5 min. PCR products were visualized on 8% polyacrilamide gel stained with ethidium bromide, as reported by Tong et al. (2002). The pBR322 DNA/MspI molecular weight marker (Tiangen) was used as standard to identify alleles.

DNA sizing and microsatellite analyses were performed with the Quantity-One software (Biorad). The analyses of polymorphism, including number of alleles (N_a), observed heterozygosity

TABLE 1. Samples of *Oncomelania hupensis* ssp. used for the construction of microsatellite-sequence-enriched library.

Subspecies	Shell sculpture	Locality (County/City, Province)	Habitat	Date	Geographical location
O. h. hupensis	Ribbed	Jiangling, Hubei	Irrigation channel	2008.09.27	30°00’31”N 112°34’43”E
O. h. hupensis	Ribbed	Jiujiang, Jiangxi	Swamp	2008.10.11	29°3’26”N 115°5’43”E
O. h. hupensis	Smooth, varix	Yushan, Jiangxi	Hilly area	2008.09.07	28°4’51”N 118°1’21”E
O. h. hupensis	Smooth, varix	Nanlin, Anhui	Hilly area	2006.05.10	30°53’18”N 118°2’27”E
O. h. robertsoni	Smooth	Xichang, Sichuan	Mountainous region	2006.06.16	27°4’04”N 102°2’10”E
O. h. robertsoni	Smooth	Dali, Yunnan	Mountainous region	2008.07.30	25°2’59”N 100°1’54”E

TABLE 2. Characterization of 41 novel polymorphic microsatellites in *Oncomelania hupensis* ssp.*

Locus	Repeat motif	Primer sequence (5'−3')	Ta (°C)	N	Size (bp)	H_O	H_E	*P* value	GenBank Accession #
Oh10	(GAA)12	F: 5'-AAATGCCCCTTGTGCCTGGAAT-3' R: 5'-TAGGACTGGTTTAGGGTTAGGGTTC-3'	58	3	136	0.5000	0.6576	0.542	GQ892883
Oh12	(ATG)22	F: 5'-CCCCACCACAACCACAAATC-3' R: 5'-GGTCGCTCAGGGTCAGAATA-3'	56	7	204	0.5667	0.6763	0.7832	GQ892884
Oh13	(TG)53	F: 5'-TGCATAAATTCCGTAGTTTT-3' R: 5'-CCCTCTTCTTCTGCCTCCTT-3'	56	4	264	0.5667	0.6808	0.0301	GQ892885
Oh14	(CA)37	F: 5'-CGTCACAAAGGAGCAACTGG-3' R: 5'-TTTACTGCTCTGCATAGCAAAGGAT-3'	56	3	274	0.5000	0.6565	0.4202	GQ892886
Oh20	(ATC)26	F: 5'-GCAGAACCCGCCAACCATTT-3' R: 5'-CAGTAGCGAGTGCTCCAACCT-3'	56	5	223	0.6333	0.7379	0.256	GQ892887
Oh30	(CT)21	F: 5'-TGTGGAAGTGGGTGGTGAGG-3' R: 5'-TGGTAAACACCGCTGTTTTTGT-3'	56	6	206	0.4203	0.5782	0.2603	GQ892888
Oh35	(AG)29	F: 5'-ATCCCATCCTATGACTATTTCCAAG-3' R: 5'-CAAACTGTAAAAGAGAGGGGTGTGT-3'	58	3	209	0.4000	0.5391	0.5721	GQ892889
Oh36	(TC)21	F:5'-AGGGTTGCTGAGGACAGAGAAAGAG-3' R:5'-CCAACTCACAACTCAGAATCCCCAT-3'	56	5	204	0.4667	0.5492	0.6475	GQ892890
Oh47	(AC)38	F: 5'-CACACGTGGTGTAGACCTACTTCTC-3' R: 5'-AACCGTTTGGGATGTAATAAATGTA-3'	58	6	218	0.4668	0.5320	0.1662	GQ892892
Oh57	(CT)59	F: 5'-GCAGCACCTCCAAACGGATTATG-3' R: 5'-GTGTGTATGTTTGTTCGGGGTTGTA-3'	56	6	318	0.6000	0.7576	0.3441	GQ892893
Oh63	(CA)32(CTT)6	F: 5'-CCGCTCCTCATCTTATCTTCTT-3' R: 5'-AAGATAGTAACCGAGAACAGTAGGG-3'	56	5	319	0.3145	0.5236	0.262	GQ892894
Oh68	(CA)36	F: 5'-AGCAGAACATTCTCAAACAAAGCG-3' R: 5'-TCGCCTCAGCTAACAGACCATCC-3'	58	4	174	0.6667	0.7571	0.4231	GQ892895
Oh69	(TG)23(CA)19	F: 5'-GCAAATACACCCAGTAACTGTCTTG-3' R: 5'-GGGCACCCTAGACAGTCCTCTGAT-3'	58	8	337	0.5340	0.5096	0.0459	GQ892896
Oh73	(TCA)24	F: 5'-ACCCTATATTTTCACTGAAGCCTCT-3' R: 5'-CATGATACGACGTCCTTTCTACAAT-3'	56	4	195	0.5333	0.6870	0.0706	GQ892897
Oh80	(CA)64	F: 5'-CCTGTAGTATGTAAGAACAATGGGT-3' R: 5'-GCGCAGTTTGGCGTGTCAGT-3'	56	3	293	0.4613	0.5433	0.047	GQ892898

(continues)

(continued)

Locus	Repeat motif	Primer sequence (5'−3')	Ta (°C)	N	Size (bp)	H_O	H_E	*P* value	GenBank Accession #
Oh94	(TG)57	F: 5'-TAGCTCTCTGATGAAACCAAATCAG-3' R: 5'-TAACGGGGGCGTAAAACAAGTC-3'	56	8	240	0.6100	0.5977	0.5021	GQ892899
Oh95	(TG)16(GA)16	F: 5'-ATAACGCCTTCCAGAAAATCAATAG-3' R: 5'-AGGGCCTTATACGGAATAAACTTGT-3'	56	6	335	0.6730	0.5301	0.0607	GQ892900
Oh100	(CA)44	F: 5'-TATATTTGAAAGCTCGGTTTGCCAT-3' R: 5'-TCCATCTGCGTACGTGTGTCTGC-3'	56	8	233	0.3446	0.4890	0.0441	GQ892901
Oh105	(ATC)62	F: 5'-GTGTCGGAAGTAGAGTAACTGTCGT-3' R: 5'-CTCCACCCTGTTTGCCTTTGC-3'	56	3	296	0.4031	0.5545	0.4463	GQ892902
Oh111	(GA)41	F: 5'-ATTGGTTGCGGCTCCAGTAT-3' R: 5'-CTTCCCTCCCCTTTCCAGAC-3'	58	4	234	0.7231	0.6043	0.0321	GQ892903
Oh125	(CT)23	F: 5'-TGGAGGCTAGGGTGAAGAAG-3’ R: 5'-GCAGACACGGAGGAGAACGA-3’	56	7	186	0.5129	0.4897	0.471	GQ892904
Oh132	(GA)43(GAA)6(ATC)17	F: 5'-GTCCAAAAGTGAGGAGAGAGGGT-3' R: 5'-CTTGATGGGAATATAAGTGGCTGT-3'	56	10	276	0.8333	0.8689	0.9617	GQ892905
Oh136	(TG)32	F: 5'-TGTTTATATTACACGCCCACCCTCT-3' R: 5'-CAGGTTCCTCCAAGTATTCATTTTC-3'	56	4	187	0.5543	0.3491	0.1965	GQ892906
Oh150	(GAA)12	F: 5'-AGGCGACACCAATTTTACATGAGG-3' R: 5'-TGTGAAATAAAGGCTTGTGGG-3'	56	6	356	0.4620	0.2135	0.56	GQ892907
Oh151	(TG)24(AG)28	F: 5'-TCACGACCAGTAATGGGG-3' R: 5'-GCAATCCTTCTTGCCTCA-3'	56	7	162	0.6803	0.5458	0.1192	GQ892908
Oh157	(CT)28	F: 5'-CTATGTTCGGATGGCAAGATTG-3' R: 5'-GACTGCGTGTAGCTTGTAAGTGC-3'	55	8	358	0.3215	0.4531	0.0651	GQ892909
Oh158	(TG)47	F: 5'-TCGTAAACAGCAAAGAGAAGAAAAG-3' R: 5'-AGTACACGCATACGCATCTACATAG-3'	56	6	251	0.4436	0.4601	0.2139	GQ892910
Oh161	(TG)16	F: 5'-CGTCCGGGCGATGGAATA-3' R: 5'-GCCAGTGAGGTTCGGTTT-3'	56	6	223	0.5792	0.7550	0.2395	GQ892911
Oh164	(TG)13	F: 5'-ACAGTCATCAGAATGTCGTTTTTAC-3' R: 5'-CAAGTAGGCATGTCAATACAAGTTA-3'	56	7	147	0.4367	0.5280	0.0941	GQ892912
Oh188	(TC)51(CA)7	F: 5'-ATAGACGAATACGCCTACATAAAAC-3' R: 5'-CGCAAAAAAGTGGGTAGACAAGAAT-3'	58	8	308	0.5870	0.5714	0.0431	GQ892913

(continues)

(continued)

Locus	Repeat motif	Primer sequence (5'−3')	Ta (°C)	N	Size (bp)	H_O	H_E	*P* value	GenBank Accession #
Oh199	(CT)12(CA)10(CT)43(CA)11	F: 5'-CTCCAACATCACGCTCCCCTACG-3' R: 5'-AAGAAAGGATTATGTGGGCATGGAT-3'	56	5	256	0.7130	0.5437	0.0453	GQ892914
Oh211	(CA)27	F: 5'-GCCACGTATTCTCCCTCTCTCCC-3' R: 5'-GGGTTCGTTCCTTTTCAGTTGCG-3'	56	6	166	0.2252	0.4301	0.771	GQ892915
Oh212	(ATC)20	F: 5'-GTTCGATCTCTTCACCACCACTAC-3' R: 5'-ACAAACGAACAACAGTAGCAATGGT-3'	56	4	215	0.5392	0.6311	0.1241	GQ892916
Oh217	(TG)46	F: 5'-TAGTGTGCAGCATAATGAGTGCA-3' R: 5'-TTATTGAGTCTCTTGACACACCTGC-3'	58	5	232	0.3000	0.5371	0.0644	GQ892917
Oh225	(CA)45	F: 5'-CTTTTTGCTCTCTCACTCGTTCTG-3' R: 5'-ATACACCTTCAAGCGTTGGTATTG-3'	58	4	210	0.4350	0.5321	0.0419	GQ892918
Oh235	(CA)39	F: 5'-TCAGATAGCCGTAACGGCAGAAAT-3' R: 5'-GCCATCAGAAACACCAAAGAGCAC-3'	58	3	272	0.6980	0.6190	0.0331	GQ892919
Oh410	(AC)31(CT)23(CA)14	F: 5'-TCAGCTTTTTGTGATGTGGTTTTT-3' R: 5'-ATGGGTTAGTGGGCATTTTTAGATT-3'	60	9	270	0.8000	0.8475	0.0701	GQ892920
Oh440	(TG)14(AG)23(GT)31	F: 5'-GGGTTAGTGGGCATTTTTAGATTTT-3' R: 5'-TTTCAGCTTTTTGTGATGTGGTTTT-3'	58	10	270	0.4667	0.496	0.0002	GQ892921
Oh448	(TC)33	F: 5'-TTCGCAGTGCCTCTACATTTCC-3' R: 5'-ATCCTCAAGCTCATTTTCTGTAACC-3'	58	6	223	0.5373	0.5689	0.9617	GQ892922
Oh500	(GTT)16(ATG)9	F: 5'-AGCTTCCATCGCCTGCTTCAC-3' R: 5'-GCAACGCTACATCACTACCCTTACT-3'	60	4	180	0.4243	0.4560	0.0587	GQ892923
Oh573-1	(TG)27	F: 5'-TGGAAGAAAAAAGAAGTGCTTATGC-3' R: 5'-GAAGTGGAAGTGAAGATGGAGGAG-3'	58	4	182	0.5937	0.6977	0.0643	GQ892924

*T_a, annealing temperature; N, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *P* value, exact test for the HWE after Bonferroni correction.

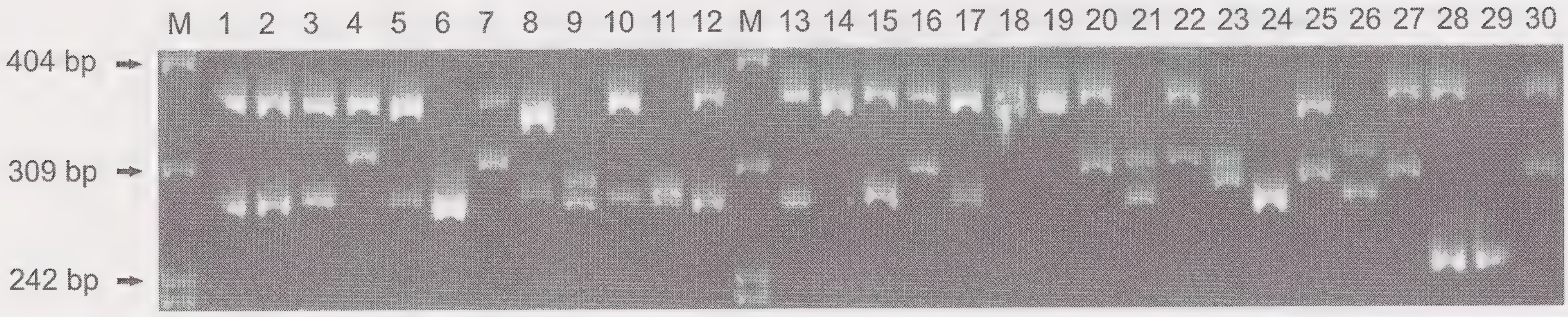
M 1 2 3 4 5 6 7 8 9 10 11 12 M 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
404 bp
309 bp
242 bp

FIG. 1. Amplification pattern of microsatellite locus Oh 132. Lanes 1–30: 30 individuals of *O. hupensis hupensis* collected from Jiangling county of Hubei province, China as listed in Table 1; M: pBR322/Msp I marker.

(H_O), expected heterozygosity (H_E), and pairwise tests for linkage disequilibrium (LD) and exact tests for the Hardy-Weinberg equilibrium (HWE) were performed by using Popgene version 1.31 (http://www.ualberta.ca/~fyeh/) and Arlequin version 3.1 (Excoffier et al., 2005; Excoffier & Heckel, 2006). The probability of occurrence of null alleles was tested using MicroChecker 2.2.3 (http://www.microchecker.hull.ac.uk/) (Van Oosterhout et al., 2004).

RESULTS AND DISCUSSION

A total of 76, out of 174 pairs of primers were proved to have specific amplification products, but only 41 primers (Table 2) deposited in GenBank (Accession # GQ892883–GQ892890, GQ892892–GQ892924) were polymorphic in the population of *O. hupensis* individuals collected from Jiangling county, Hubei province (Fig. 1). It was also proved that these 41 primers were polymorphic in samples obtained from Anhui, Jiangxi, Sichuan and Yunnan Province (data not shown).

For the samples obtained from Jiangling county, Hubei province, the number of alleles per locus ranged from three to ten with an average of 5.5, and the observed heterozygosity, *H*o and expected heterozygosity, *H*e, ranged from 0.2252 to 0.8333 (average 0.5105) and 0.2135 to 0.8475 (average 0.5704), respectively (Table 2). These loci were shown to be polymorphic. When the *H*o and *H*e value scale of alleles was higher, polymorphism of locus was more obvious, for example Oh132. All pairwise tests for linkage disequilibrium among loci and exact tests for the HWE were non-significant after applying sequential Bonferroni correction ($P < 0.05$). Using the software MicroChecker showed that there were not null alleles at all loci.

In conclusion, novel polymorphic microsatellite sequences have been successfully identified in the intermediate host, *Oncomelania hupensis*, of *Schistosoma japonicum* in the present study. It has been further proved that these microsatellite markers can be employed effectively to examine the population variations at least in the so far confirmed two subspecies, *O. hupensis hupensis* and *O. hupensis robertsoni* (Davis et al., 1995, 1998). However, it may be also of great interest if these microsatellite markers can be used to detect other sub-species of *Oncomelania hupensis*, such as *O. hupensis tangi* and *O. hupensis guangxiensi* at population level.

ACKNOWLEDGEMENTS

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THE HIGHEST SOUTHERN LATITUDE RECORD OF A LIVING *TRIDACNA GIGAS*

Luke C. Strotz[1*, 2], Briony L. Mamo[2] , Timothy P. Topper[2] & Collette Bagnato[3]

INTRODUCTION

Tridacna gigas (Linnaeus), growing to over 1 m in length and weighing up to 200 kg (Lucas, 1988), is the largest extant representative of the Bivalvia. This familiar and charismatic taxon, found throughout the Pacific region, is a well recognized, important and iconic component of coral reef communities (Rosewater, 1965; Yonge, 1981). Despite its iconic status, the species is under threat due to over-harvesting and habitat destruction, and is listed as "vulnerable" on the IUCN's red list of threatened species (IUCN, 1983; Juinio et al., 1989; Hviding, 1993; Munro, 1993; Lucas, 1994).

Like hermatypic scleractinians, *T. gigas* has a symbiotic relationship with photosynthetic dinoflagellate algae, and it is this relationship that has allowed it to obtain its huge size (Schneider, 1998). Because of this similarity to corals, members of the Tridacninae, including *T. gigas*, have been used as a proxy to assess the health of coral reef settings (Aharon, 1991; Pätzold et al., 1991) and for reconstructing paleoclimates at a high-resolution (Ahron, 1983, 1991; Aharon & Chappell, 1986; Watanabe & Oba, 1999). Previous paleoclimate studies have concentrated on shell chemistry, as *T. gigas* calcifies in isotopic equilibrium with surrounding seawater (Watanabe et al., 2004). However, as temperature is a major control on distribution, with previous studies determining that shell growth is impossible below 19.2°C and optimum growth occurs in regions where the temperature is above 25°C for much of the year (Lucas et al., 1989), presence/absence of the taxon can also be an indicator of paleotemperature.

The above factors make understanding the modern distribution of *T. gigas* vitally important, as it has implications for conservation of the species and both current and future studies of the effects of changing climate on tropical reef ecosystems. Given the strict ecological control on its distribution (temperature limited), *T. gigas* is an obvious candidate to identify and evaluate the possible influence of changing climate on coral reef communities.

MATERIAL AND METHODS

Previously Known Distribution

The geographic range of *T. gigas* is poorly constrained, and the southern extent of the species is unknown. Based upon the distribution map provided by Lucas (1988: 22) the southernmost occurrences of *T. gigas* occur on the Great Barrier Reef and its distribution should extend below the Tropic of Capricorn (23°26.366'). However, Lucas (1988) provides no specific occurrences and notes that the species is "nearly extinct or recently extinct over a considerable part of the range". Most occurrences recorded in the literature for the Great Barrier Reef are largely confined to the northern portion of the reef (less than 20°S) with few records of this taxon at latitudes greater than 19°S (Fig. 1). Braley (1987a, b), reported no living individuals south of 19°33'S in a census of *T. gigas* populations on the Great Barrier Reef. Ayling & Ayling (1986), in their survey of biodiversity of the southern Great Barrier Reef, recorded *T. gigas* in the north-easterly part of the Swain Reefs and at Elusive Reef (21°4.777'S). They specifically list *T. gigas* as absent from sampled sites in the southern-most part of the Great Barrier Reef, such as Heron Island and other Capricornia section reefs (Ayling & Ayling, 1986, Table 13). The Global Biodiversity Information Facility (GBIF) database lists no records of *T. gigas* south of 21°44'S and all of the southern-most specimens recorded represent single disarticulated valves of deceased individuals of indeterminate age (I. Loch pers. comm.) Therefore, no records exist in the literature of live individuals in the southern part of the Great Barrier Reef, south of the Capricorn Channel.

Location

Heron Reef, part of the Capricorn-Bunker group, is located just south of the Tropic of Capricorn, and 85 km north-east of Gladstone, central

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[1] School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia
[2] Department of Biology, Macquarie University, Sydney, NSW 2109, Australia
[3] The Centre for Marine Studies, The University of Queensland, Brisbane, QLD 4072, Australia
*Corresponding author: l.strotz@unsw.edu.au

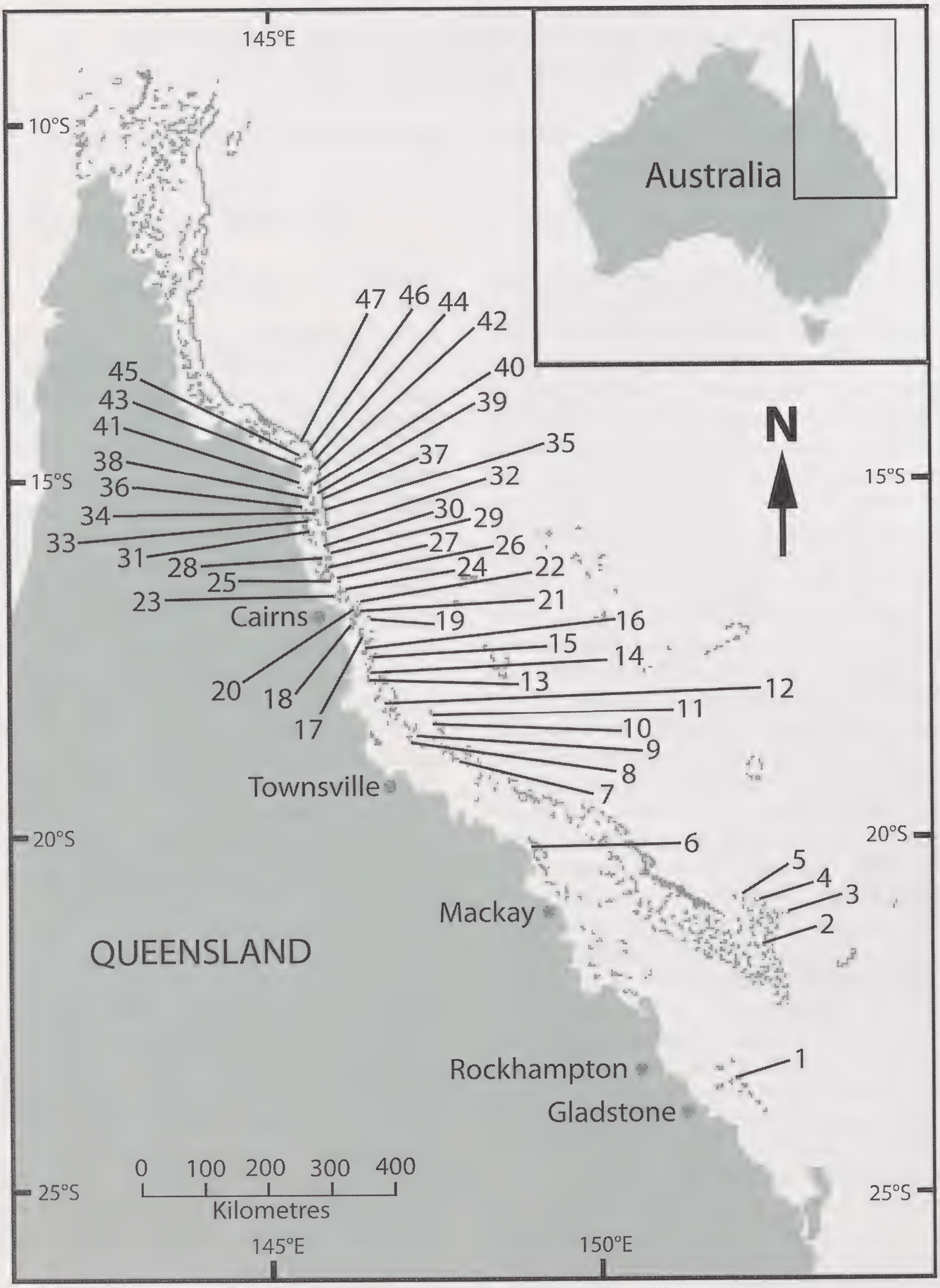


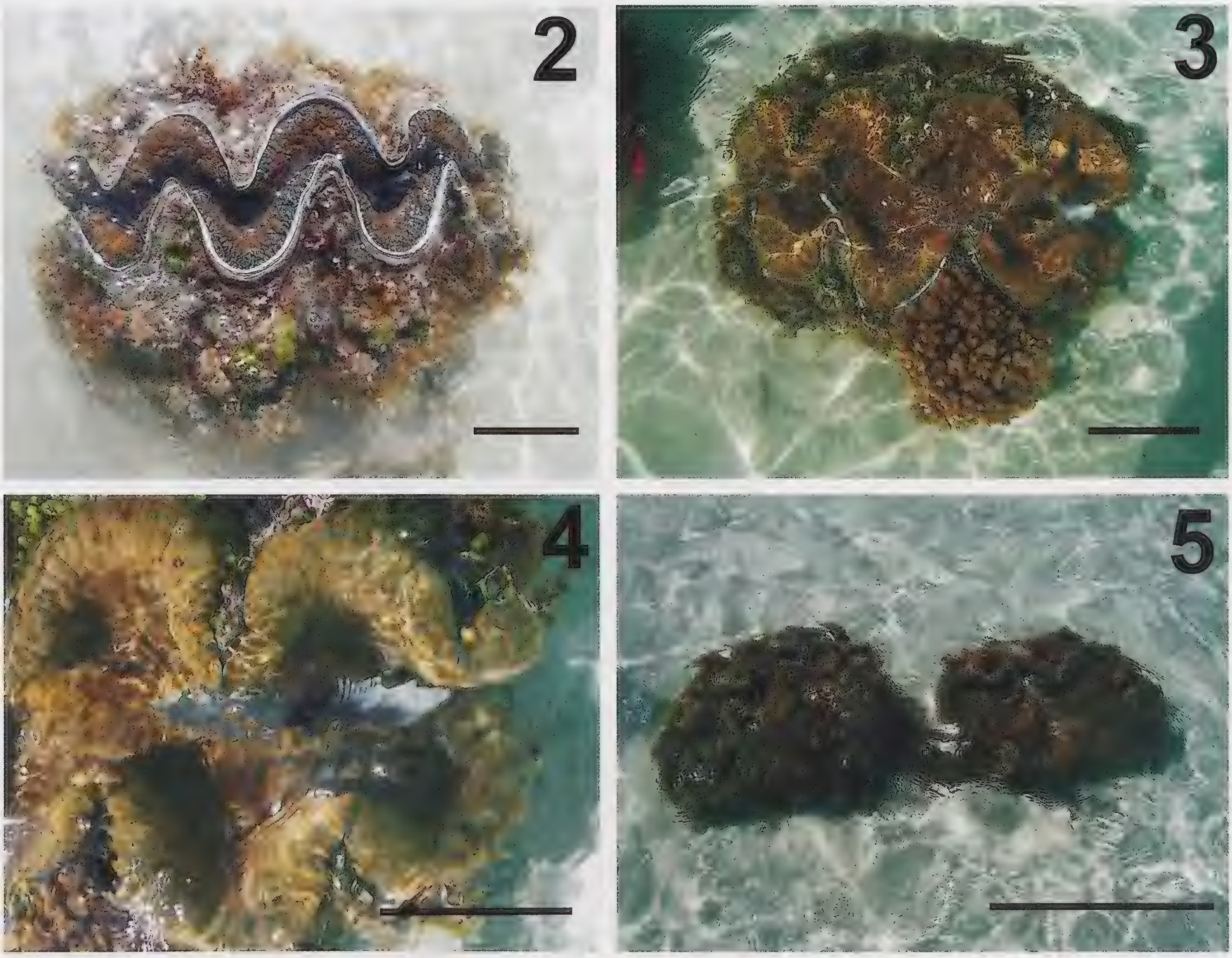
FIG. 1. Occurrences of *Tridacna gigas* on the Great Barrier Reef, based upon data published by Braley (1984), Ayling & Ayling (1986), Braley (1987a, b), Global Biodiversity Initiative Fund Portal (accessed 2008) and this study. 1. Heron Island, 2. Frigate Cay, 3. Elusive Reef, 4. 20-396 Reef, 5. 20-394 Reef, 6. Hayman Island, 7. Broadhurst Reef, 8. Lodestone Reef, 9. John Brewer Reef, 10. Dip Reef, 11. Myrmidon Reef, 12. Brook Island Reef, 13. Barnard Island, 14. Feather Reef, 15. Wardle Reef, 16. Peart Reef, 17. Russell Island, 18. High Island, 19. Channel Island, 20. Fitzroy Island, 21. Northwest Reef, 22. Flynn Reef, 23. Green Island, 24. Arlington Reef, 25. Upolu Reef, 26. Michaelmas Reef, 27. Batt Reef, 28. Opal Reef, 29. Tongue Reef, 30. St Crispin Reef, 31. Hope Island, 32. Escape Reef, 33. Cowlishaw Reef, 34. 15-043 Reef, 35. Ribbon Reef No. 5, 36. Egret Reef, 37. Ribbon Reef No. 6, 38. Boulder Reef, 39. Williamson Reef, 40. Forrester Reef, 41. Low Wooded Island, 42. Two Island, 43. Martin Reef, 44. Macgillivray Reef, 45. Lizard Island, 46. Carter Reef, 47. Day Reef.

Queensland, Australia. Heron Reef is approximately 27 km^2 in area, with Heron Island, a carbonate sand cay, located at the western end. A central lagoon dominates much of the reef (17 km^2) with the remainder of the reef consisting of shallow-water reef flat. Ambient water temperature varies between 20°C to 26°C.

RESULTS AND DISCUSSION

In April 2008, during a survey of the southern Heron Island reef flat, part of the Capricorn-Bunker group, four living specimens of *T. gigas* were discovered at 23°26.778'S. These specimens had been previously noted by one of the authors (Bagnato), who was unaware of their significance, and so had not officially recorded them. These specimens are the southernmost specimens of *T. gigas* ever recorded.

The Heron Island specimens are assigned to *T. gigas* based upon their large size, between 71 and 80 cm in length. This is well in excess of the maximum size of the next largest member of the genus *Tridacna*, *Tridacna derasa*, which reaches shell lengths of up to 60 cm (Lucas, 1988). The Heron Island specimens also exhibit shell thickening at the umbo, have a brown mantle with blue-green eyespots and have elongate, triangular projections present along the upper margin of the valve, all features that characterize *T. gigas* (Lucas, 1988) (Figs. 2–5).



FIGS. 2–5. *Tridacna gigas* individuals found at Heron Island. Orientations based upon Yonge (1953). FIG 2: Ventral view of *T. gigas* from Heron Island. Note the large size (see scale bar) and triangular projections present along the upper margin of the valve that are characteristic of the species (Lucas, 1988); FIG. 3: Ventral view of *T. gigas* from Heron Island. Note the *Stylophora* sp. colony growing on the shell. Based upon growth rates for *Stylophora pistillata* calculated by Loya (1976), this colony is between 12 and 14 years old; FIG. 4: Detail of Fig. 3, showing close-up of mantle, eyespots and inhalant siphon; FIG. 5: Two *T. gigas* from Heron Island growing in close proximity to one another. Scale bars are based upon measurements made in the field. Scale bars Fig. 2 = 20 cm; Fig. 3 = 10 cm; Fig. 4 = 20 cm; Fig. 5 = 70 cm.

All four individuals are located within a small (~12 m) area on the inner reef flat. The size of the Heron Island specimens is comparable to other adult individuals from the northern Great Barrier Reef (Ayling & Ayling, 1986; Braley, 1987a, b). Based upon growth rates for *T. gigas* calculated by Pearson & Munro (1991) the age of the Heron Island specimens is estimated at between 25 and 38 years. However, Pearson & Munro (1991) note that growth rates will be greater in warmer waters. Given that the sea surface temperatures at Heron Island are, on average, 2°C cooler than localities studied by Pearson & Munro (1991) (Lough, 1999), it is highly possible that the recorded specimens are much older.

The presence of *T. gigas* on Heron Island reef flat is surprising, given that sea surface temperatures in the area reach as low as 18°C (Lough, 1999), well below the temperature proposed for shell growth (Lucas et al., 1989). This is not the coldest water occurrence of the taxon, with higher latitude specimens of *T. gigas* recorded from Okinawa (Matsukuma, 2000), where sea surface temperatures reach as low as 17.5°C (Kanak & Tachihara, 2006). For over seven months of the year however, sea surface temperatures at Heron Island are below 25°C (Lough, 1999), much longer than at Okinawa (Kanak & Tachihara, 2006), and during the remaining five months, exceed 25°C for only very short periods (Lough, 1999). Previous work has proposed that 25°C is the optimum growing temperature for *T. gigas* (Lucas et al., 1989), and therefore the size of the Heron Island specimens would suggest that *T. gigas* is capable of growth in regions where average sea surface temperatures are lower than has been previously proposed.

As *T. gigas* can reach full sexual maturity in as little as four years (Fitt, 1991), and based upon the estimated ages of the Heron Island specimens, all four specimens are sexually mature. It is unclear, however, whether any of the four specimens have ever spawned. Fitt & Trench (1981) determined that spawning is not linked to temperature. Therefore, the comparatively cooler sea surface temperatures at Heron Island (Lough, 1999) should not preclude the Heron Island specimens from spawning. Searches of the reef flat both up-current and down-current of their location found no specimens of *Tridacna*, either juvenile or adult, that could be assigned to *T. gigas*. One of the authors (Bagnato) has conducted thousands of surveys of both Heron reef and lagoon over a period of decades and has never observed any other specimens of *T. gigas,* other than those recorded in this study. This suggests either the Heron Island specimens have never spawned, or that no viable offspring have been produced due to the small size of the population.

With no other specimens recorded or observed from the Heron Island Reef, either from reef flat or lagoonal settings, and no records of *T. gigas* from any other reefs in the Capricon-Bunker group, this would indicate two possibilities. Either the Heron Island specimens represent a remnant population or that that the source of the Heron Island *T. gigas* specimens is elsewhere. The authors recall that a single valve of *T. gigas* was once part of a display at the Heron Island Research Station and was supposedly obtained during construction of the Heron Island harbour in the 1960s. This potentially supports the possibility that a larger *T. gigas* population was once present on Heron Island, but with the current limited evidence it is impossible to be definitive.

If the Heron Island specimens are sourced from elsewhere, the closest live specimens of *T. gigas* recorded are from the northeastern part of the Swain Reefs (Ayling & Ayling, 1986), approximately 300 km north of Heron Island, and represent the most likely source. This trip includes crossing the Capricorn Channel, almost 120 km of open water that divides the southern Great Barrier Reef into two geomorphologically distinct reef systems (Kleypas & Burrage, 1994) and, at 200 m deep (Bostock et al., 2009), represents a distinct ecological boundary. As *T. gigas* reaches the pediveliger stage between 10–29 days after fertilization (Fitt & Trench, 1981), after which it becomes largely confined to the benthic zone, the Heron Island *T. gigas* specimens could potentially make this journey as part of the plankton. If true, this has implications for understanding genetic diversity and population dynamics of a variety of taxa in the southern Great Barrier Reef setting, as it suggests that free-swimming larvae produced by populations from Swain Reefs are able to colonize sites in the southernmost portion of the Great Barrier Reef, assuming they can survive the journey.

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HIGH PHENOTYPIC FREQUENCIES OF COMPLETE ALBINISM IN WILD POPULATIONS OF *BIOMPHALARIA PFEIFFERI* (GASTROPODA: PULMONATA)

Gabriel Mouahid[1]*, Rodrigue Mintsa Nguema[1], Mohamed A. Idris[2], Mahmoud A. Shaban[3], Salem Al Yafee[3], Juliette Langand[1], Marion Verdoit-Jarraya[1], Richard Galinier[1] & Hélène Moné[1]

Complete albinism in wild animal populations is a rare condition. It is characterized by the lack of melanin in both the integument and eyes. Consequently, there are few reports on this type of albinism. The phenotypic frequency ranges from 0.00007 to 0.0002 in humans (Africans) (Hong et al., 2006) and is 0.002 in the cave-dwelling bat, *Hipposideros diadema nicobarensis* (Aul & Marimuthu, 2006). In amphibians, frequency ranges from 0.02 to 0.03 in the cavern salamander *Gyrinophilus porphyriticus* (Brandon & Rutherford, 1967), and is the highest (0.12) in the chorus frog *Pseudacris triseriata* (Corn, 1986).

In wild populations of freshwater snails, complete albinism has been observed, but its frequency has been recorded only rarely. Yusa (2004) collected 2,000 snails of *Pomacea canaliculata* in lotus fields and adjacent canals in Kumamoto, Japan, and found that 28 (1.4%) were albino (yellow). The frequency (0.014) of this phenotype remained constant for six years in the collection area. Madsen et al. (1983) reported the existence of "a few" albinos in *Helisoma duryi* during a malacological survey in Bamburi, Kenya. Dillon & Wethington (1992) found one *Biomphalaria obstructa* and two *Physa heterostropha* albino snails "among thousands of individuals" collected in South Carolina.

Researchers who have used albino strains of freshwater snails for genetic crossing experiments with melanic strains or for host-parasite compatibility studies have only reported the origin of the albino strain, such as Richards (1962) for *Biomphalaria glabrata* from Venezuela, Boissier & Moné (2001), and Boissier et al. (2003) for *B. glabrata* from Brazil, Rosa et al. (2004) for *Biomphalaria tenagophila* from Brazil, and Davis & Ruff (1973) for *Oncomelania hupensis* from southern Taiwan. Most genetic studies using albino snails have been performed with albino individuals that originated as "spontaneous mutations" from laboratory-maintained snail colonies. These animals were then isolated and established for experimental purposes. The well-known albino strain of *B. glabrata* that originated in the laboratory of Newton (1954, 1955) has been used for numerous studies. This strain has been used widely for genetic analyses (Richards, 1969, 1973) and for genetic studies on compatibility with *Schistosoma mansoni* (Richards & Merritt, 1972; Mulvey & Woodruff, 1985), the causative agent of human intestinal bilharziasis. Other laboratory-discovered albino snails include *Lymnaea peregra* (Boycott & Diver, 1927) and *Oncomelania formosana* (Moose & Suzuki, 1964).

The present paper is on the phenotypic frequencies of complete albinism in wild populations of *Biomphalaria pfeifferi* (Fig. 1), the intermediate host of *S. mansoni* in Africa and the Middle-East (Dejong et al., 2003). The relationship between the condition of albinism and several environmental factors related to water quality were tested.

This study was done in the Dhofar region in southwestern Oman near Yemen (Fig. 2) where bilharziasis is reemerging (Idris et al., 2003; Moné et al., 2003). The area of study forms a wide band 250 km long and 30 km wide (7,500 km^2) along the Dhofar coast and includes three drainage basins that empty into the Arabian Sea (Figs. 3–5). Fourteen sites harboring *B. pfeifferi* were surveyed for the snails nine times between November 2000 and November 2007 (Figs. 3–5). These sites are situated in spring-fed rivers, and most of them are isolated from each other without any

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[1] Laboratoire de Biologie et d'Ecologie Tropicale et Méditerranéenne, UMR 5244 CNRS-EPHE-UPVD, Université Via Domitia de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan, France

[2] Department of Microbiology & Immunology, College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, Oman

[3] Sultan Qaboos Hospital, Directorate General of Health Services, Dhofar Governorate, Salalah, Oman

*Corresponding author: mouahid@univ-perp.fr

confluence in the valleys (Table 1). They were surveyed at least three times.

Snails were collected with a stainless steel strainer (18 cm in diameter, 8 cm deep, 20 cm handle, and 1 mm pore size) attached to a wooden handle 140 cm long. The snails were transported to the laboratory in Salalah for identification using anatomical features (Ibikounlé et al., 2008). The frequencies of albinism were determined at the time of each survey. The GPS position (longitude and latitude) and altitude of each site were determined (Table 1). Water quality at these sites was defined once in May 2007 by eight parameters: temperature (°C), oxygen (mg/L), pH, nitrite (mg/L), nitrate (mg/L), ammonium (mg/L), phosphate (mg/L) and total hardness (mg/L) using Compact Laboratory Merck KGaA (64271 Darmstadt, Germany). All the data are recorded in Table 1. A principal component analysis centred and standardized was performed using R version 2.4.0 software. The non-parametric Kruskall-Wallis test was performed on the environmental data using SPSS version 10.0.5 software.

The total number of *B. pfeifferi* snails collected in the study was 16,613. Albino snails were found in only two out of the 14 sites: in Siginitti, from May 2001 onwards, and in Tibraq only in May 2007 (Table 2). These two sites are isolated from each other (Fig. 4). The principal component analysis showed that none of the environmental parameters was associated with the presence of albinism. The first and second axes explained only 24.8 and 20.6% of variance, respectively. There was no significant difference in the environmental parameters ($P > 0.05$) between the two sites harbouring albino snails and the other 12 sites.

In both Siginitti and Tibraq, albino and melanic snails coexist and are exposed to

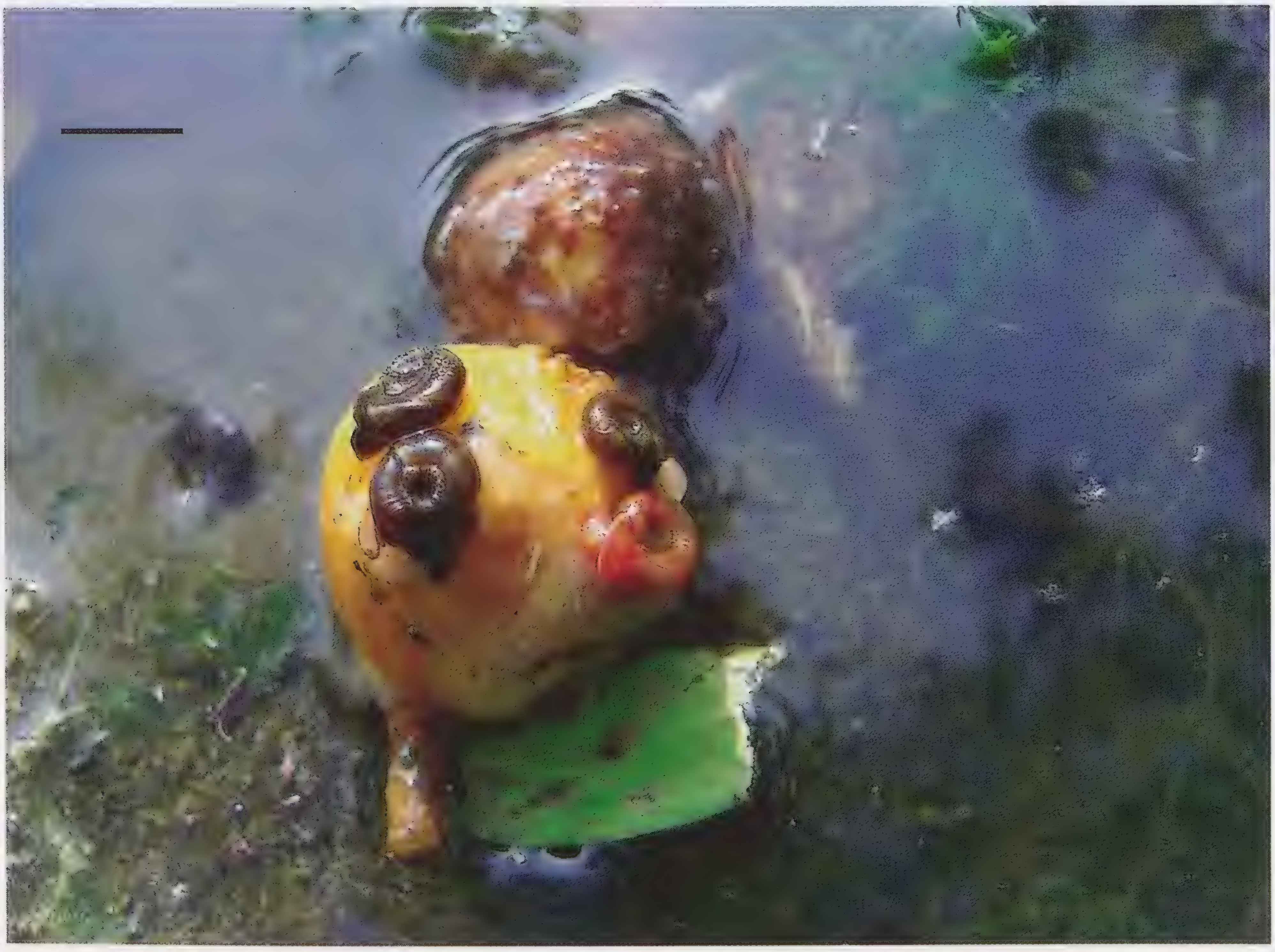


FIG. 1. Albino and pigmented *Biomphalaria pfeifferi* feeding on a fig in natural conditions in Dhofar (Oman). Scale bar = 8 mm.

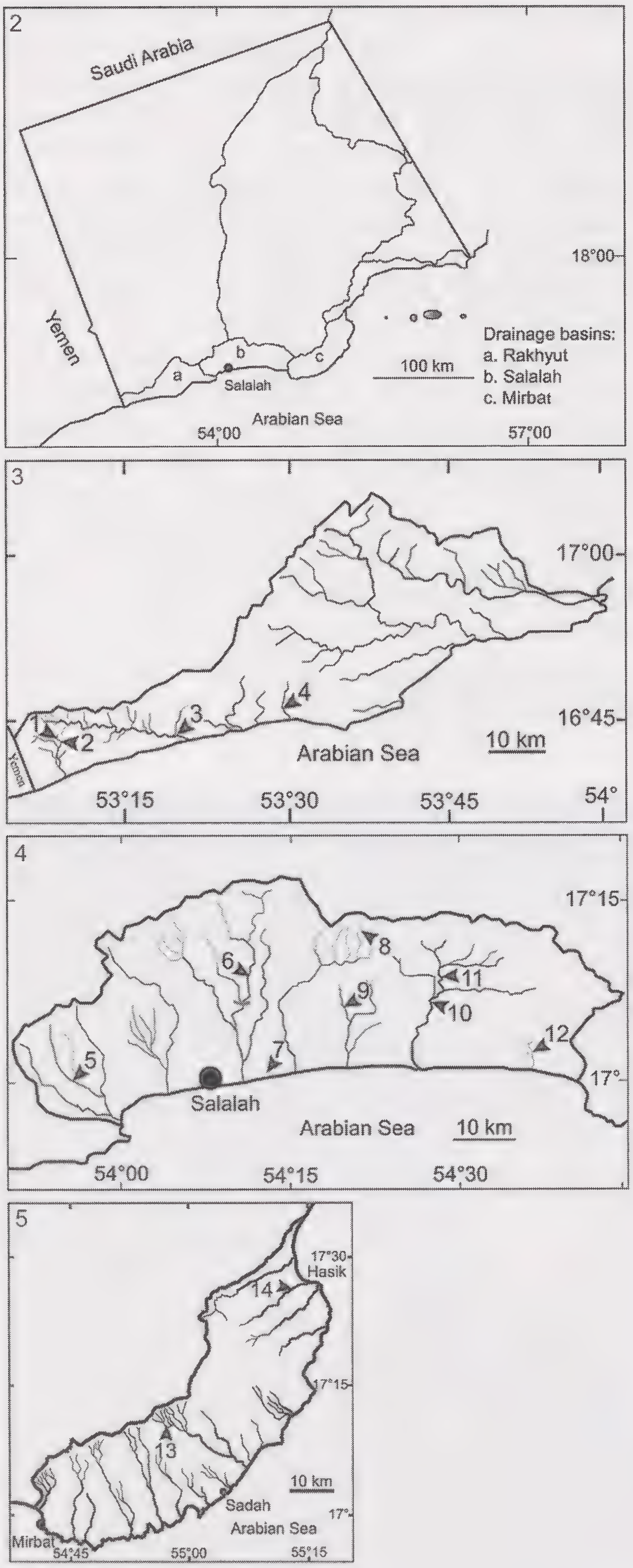
TABLE 1. Name, longitude, latitude, altitude, and eight water testing parameters of the surveyed sites harbouring *Biomphalaria pfeifferi* in Dhofar, Oman. *, ** and *** show the sites that are situated in the same valley. In bold, the two sites harbouring albino snails.

Name	Longitude (East)	Latitude (North)	Altitude (m)	Temperature (°C)	O_2 (mg/L)	pH	Nitrite (mg/L)	Nitrate (mg/L)	Ammonium (mg/L)	Phosphate (mg/L)	Total Hardness (mg/L)
Sarfait*	53°08’13.99”	16°41’47.69”	636	27	9.1	8.00	0.025	10	0.00	0.00	570
Kohood*	53°08’14.83”	16°42’17.99”	818	30	6.6	7.50	0.050	10	0.00	0.00	350
Kharfut	53°19’41.43”	16°44’02.84”	41	31	13.2	7.50	0.000	10	0.50	0.00	380
Hotah	53°30’44.71”	16°45’50.37”	57	31	9.9	8.00	0.000	0	0.00	0.00	370
Sheitt	53°49’06.54”	17°00’01.47”	221	31	6.2	8.00	0.000	0	0.00	0.15	370
Sahalnout	54°10’43.02”	17°08’53.64”	200	32	8.4	7.50	0.000	10	0.00	0.00	315
Arazat farm**	54°12’58.86”	17°02’14.09”	22	30	9.1	8.25	0.200	0	0.15	0.00	230
Birin**	54°19’37.31”	17°13’35.94”	793	27	11.4	8.25	0.500	50	0.20	0.10	350
Tibraq	54°19’35.09”	17°05’58.11”	101	29	16.3	8.00	0.025	10	0.00	0.00	280
Wadi Derbat***	54°27’12.09”	17°06’12.57”	201	36	10.0	8.00	0.060	10	0.00	0.00	310
Siginitti***	54°28’08.99”	17°08’36.44”	334	29	10.3	7.50	0.500	10	0.00	0.10	370
Heisher	54°36’29.17”	17°03’14.58”	286	31	9.7	8.00	0.000	0	0.00	0.00	350
Lagga-Shalyun	54°56’29.12”	17°11’20.34”	467	29	7.6	8.50	0.000	0	0.50	0.10	265
Had Barm	55°14’36.42”	17°26’32.14”	33	26	6.5	7.50	0.000	0	0.00	0.00	520

the same environmental conditions, such as water velocity, flooding events, temperature, and photoperiod. In Siginitti, albinos were found in 2001 at a frequency of 0.079 and then increased to 0.412 in 2005 and to 0.491 in 2006 (Table 2). In 2007, the frequency increased slightly to 0.494 in May and then dropped significantly to 0.184 in November. In 2003 and 2004 surveys were not possible at Siginitti because a flood in May 2002 washed away the only accessible road to the study site. In Tibraq, albinos were found in May 2007 at the frequency of 0.075 but were no longer present five months later (Table 2). The frequency of the albino allele was inferred for each survey using the observed phenotypic frequencies and the lowest known value of selfing rate for *B. pfeifferi*, 0.787 (Charbonnel et al., 2002). The calculated values of albino allelic frequencies are slightly higher than the observed frequencies presented (Table 2).

This is the first report of the presence of albinism in wild populations of *B. pfeifferi*. The observed phenotypic frequencies close to 0.50 are the highest ever recorded for wild populations of snails. Prior to this study, the highest frequency of 0.12 was found in the frog *P. triseriata* in which the survival rate of the embryos and growth of the juveniles was the same as the melanic phenotype (Corn, 1986).

We found that albino snails in the wild can be present at high frequencies (> 0.40) for extended periods of up to 20 months. With a generation time of three months, this represents about six reproductive cycles. In this study, the condition of albinism could not be correlated to water quality as defined by measurements of 8 parameters. In order to understand the causes of temporal variations in the phenotypic frequencies of albinism, the effects of genetic drift, migration and natural selection need to be evaluated.



FIGS. 2–5. Maps of Dhofar Governorate. FIG. 2: General view of the three drainage basins (a: Rakhyut basin, see Fig. 3; b: Salalah basin, see Fig. 4; c: Mirbat basin, see Fig. 5); FIGS. 3–5: Exact positions (arrows with numbers) of each of the 14 surveyed points in each of these three drainage basins. 1: Sarfait; 2: Kohood; 3: Kharfut; 4: Hotah; 5: Sheitt; 6: Sahalnout; 7: Arazat farm; 8: Birin; 9: Tibraq; 10: Wadi Derbat; 11: Siginitti; 12: Heisher; 13: Lagga-Shalyyun; 14: Had Barm.

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TABLE 2. Observed phenotypic (Obs. F) and expected (estimated) allelic (Exp. F) frequencies of complete albinism in *Biomphalaria pfeifferi* in Siginitti and Tibraq sites (Dhofar, Oman); n = number of snails collected.

	Siginitti			Tibraq		
Month, Year	Obs. F	Exp. F	n	Obs. F	Exp. F	n
November 2000	0.000	0.000	453	0.000	0.000	89
May 2001	0.079	0.114	229	0.000	0.000	664
February 2002	0.051	0.075	474	0.000	0.000	553
September 2002	-	-	-	0.000	0.000	150
December 2003/January 2004	-	-	-	0.000	0.000	667
September 2005	0.412	0.500	578	0.000	0.000	328
February 2006	0.491	0.577	407	0.000	0.000	51
May 2007	0.494	0.580	162	0.075	0.110	623
November 2007	0.184	0.250	620	0.000	0.000	272

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EGG HULL MORPHOLOGY IN TWO CHITONS (POLYPLACOPHORA) FROM THE SOUTHWESTERN ATLANTIC OCEAN

Cristián Ituarte*, María Gabriela Liuzzi & Romina Centurión

Museo Argentino de Ciencias Naturales – CONICET,
Av. Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina

INTRODUCTION

Polyplacophorans with planktotrophic development (free spawners) are known to have eggs with elaborate extra-cellular coverings known as egg hulls (Eernisse & Reynolds, 1994). In these species, hulls are commonly ornamented with projections in the form of cupules, cups, cones, flaps or spines. Members of Lepidochitonidae (*Lepidochitona* spp., now classified as *Cyanoplax* spp.) from the Pacific coast of North America were shown to have reduced, nearly smooth, egg hull sculpturing, which is a reduction of a cone-like hull in free-spawning relatives (Eernisse, 1988). *Leptochiton asellus*, a species of Leptochitonidae (Lepidopleurida) has eggs with smooth jelly-like hulls (Buckland-Nicks & Hodgson, 2000; Buckland-Nicks, 2008). Some brooder species, such as *Chiton nigrovirescens*, have eggs with hulls provided with short spines that serve to maintain eggs packed within the pallial groove (Buckland-Nicks & Brothers, 2008). Thus, the shape and the length of hull projections is indicative of the mode of development, that is, brooders vs. free spawners (Eernisse, 1988).

Variations in the morphology of the egg hulls are known to be of taxonomic value (Eernisse, 1984; Sirenko, 1993; Eernisse & Reynolds, 1994). Recently, the morphology of hull projections was recognized as a valuable character in the study of phylogenetic affinities (Buckland-Nicks, 2006, 2008; Sirenko, 2006).

During oogenesis, follicle cells are probably responsible for the shaping of the egg hulls, acting as molds for the depositing of mucopolysaccharides and proteins that constitute the egg hull layers (Eernisse & Reynolds, 1994). Buckland-Nicks & Reunov (2009) reported the involvement of both oocyte and follicle cells in the formation of the egg hull in a chitonid, *Rhyssoplax tulipa*.

The existence of a close relationship between the shape of egg hulls, morphology of sperm, and mode of fertilization has been reported (Buckland-Nicks, 1993, 1995, 2006, 2008). All chiton species with complex egg hulls have dart-like sperm with reduced acrosomes, usually at the tip of a nuclear filament of variable length; conversely, lepidopleurid species with smooth egg hulls have sperm with prominent acrosomes (Hodgson et al., 1988; Pashchenko & Drosdov, 1998; Buckland-Nicks, 2006; Buckland-Nicks & Brothers, 2008).

Knowledge of the hull morphology and characteristics of the follicle epithelium of genera and species from the southwestern Atlantic is scant. Sirenko (1993, 2006) provided general sketches of the hull morphology of many chitons, among them, species of the genus *Chaetopleura*, *Plaxiphora* and *Tonicia*. However, details of the morphology of these species are entirely unknown. Buckland-Nicks (2008) and Buckland-Nicks & Brothers (2008) reporting on the fertilization process and evolution in chitons, briefly described and illustrated the hull morphology of species of the genera *Chaetopleura* and *Callochiton* from the Northern Hemisphere.

The present study describes the ultrastructure of the egg hulls of *Chaetopleura isabellei* (d'Orbigny, 1841) and *Plaxiphora aurata* (Spalowsky, 1795), two common species along Argentine shores. A peculiar behavior of the ooplasm during early stages of oogenesis in *P. aurata* is described. This characteristic, to date reported only in species belonging to Acantochitonina, could represent a reproductive trait unique for this clade, reinforcing the phylogenetic coherence derived from morphology. Our results suggest that knowledge of details of the oogenesis process, a dramatic event in defining life history of a species, may be of help in defining phylogenetic relationships among chitons. Morphology of the egg hull of *P. aurata* here reported, strongly suggests the need for a new placement of *Plaxiphora*, outside Mopaliidae.

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*Corresponding author: ituarte@retina.ar

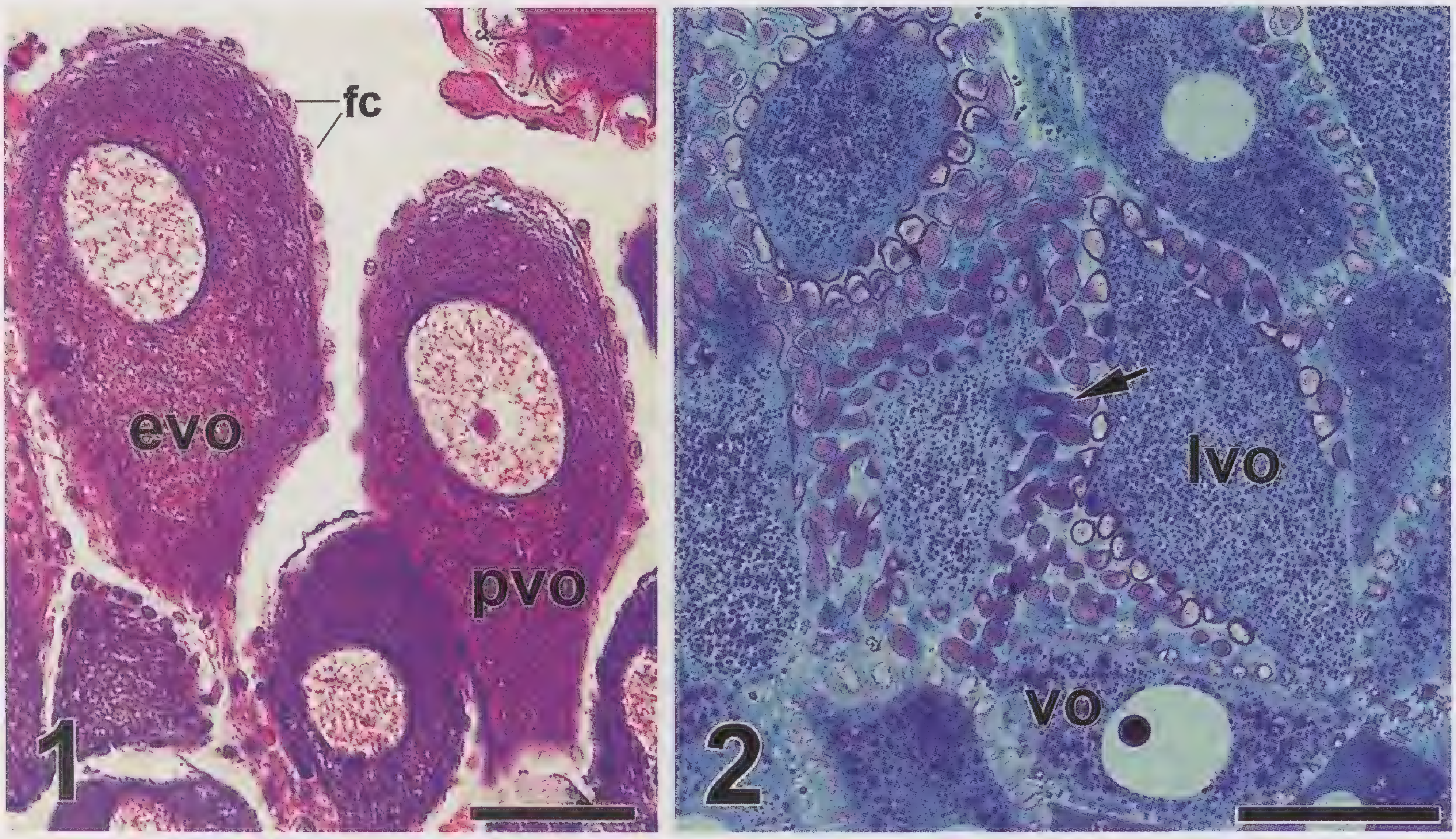
MATERIALS AND METHODS

Specimens of *Plaxiphora aurata* were collected on July 2008 from the rocky shore at Punta Cuevas (42°46'46"S, 65°00'06"W), Chubut, Argentina, and processed immediately for histology and scanning electron microscopy (SEM). Specimens of *Chaetopleura isabellei* were collected at Necochea (38°34'S, 58°39'W), Buenos Aires, Argentina, and maintained in aquaria with aerated sea water, exposed to a light/dark (L/D) cycle of 24 h (L/D: 12/12). On January 5, 2009, ripe eggs and sperm coming from spontaneous gamete emissions in the aquaria were collected in small vials and fixed in a 5% formaldehyde solution. After dehydration in ascending ethanol series, in place of crytical point drying, eggs and sperm were treated for 5 min in hexamethyldisilazane, air dried, mounted in stubs and coated for SEM. Female gonads of *P. aurata* and *C. isabellei* were fixed in 4% glutaraldehyde with 5% formaldehyde solution in phosphate buffer (pH 7.1) at 4°C for 20 h. A portion of gonads and free oocytes were dehydrated and treated as previously described for SEM. Gonads for general histology were infiltrated in Historesin® (Leica) and sectioned in a rotary motorized Leica RM 2265 microtome. Sections of 3.5 µm were stained either with haematoxylin-eosin or toluidine blue (pH 9.0).

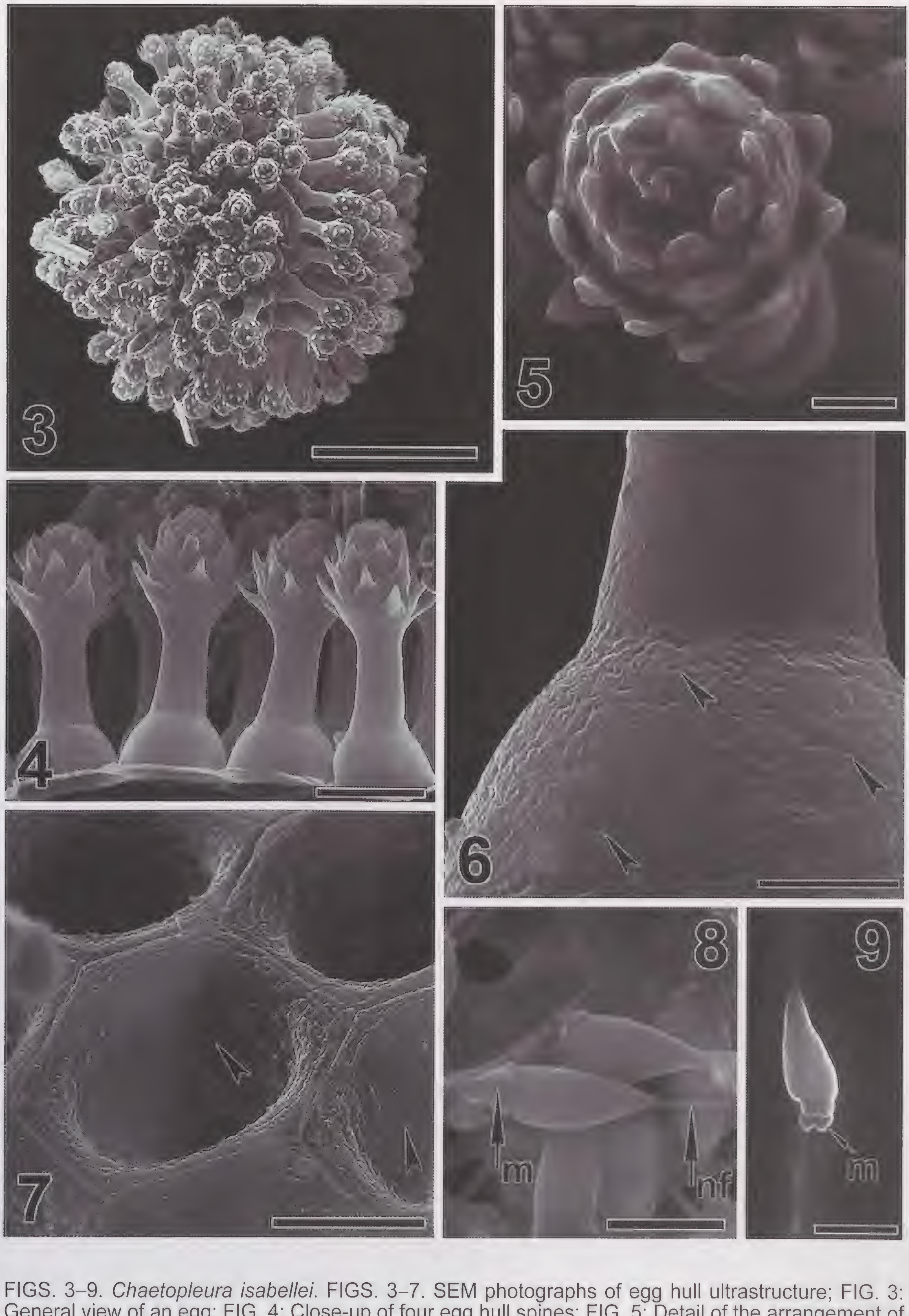
RESULTS

Chaetopleura isabellei

Just before vitellogenesis begins in *Chaetopleura isabellei* (Chaetopleuridae: Chitonina), each oocyte is completely surrounded by a large number of follicle cells (more than 30 per transverse section), closely applied to the plasma membrane (Fig. 1). As oogenesis progresses, the deposition of materials forming the egg hull begins, and the follicular epithelium is progressively pushed away from the oocyte surface (Fig. 2). Under light microscopy, hull projections appear composed of two layers of materials when stained with toluidine blue and viewed with light microscopy; an outer, very thin layer showed an orthochromatic reaction, and an inner layer was metachromatic. These reactions are compatible with neutral and acid mucopolysaccharides, respectively (Fig. 2). Ripe eggs were about 295 µm diameter (after dehydrating for SEM egg diameter was reduced



FIGS. 1, 2. Oogenesis in *Chaetopleura isabellei*. FIG. 1: Previtellogenic oocyte (pvo) and early vitellogenic oocytes (evo) (note acidophilic droplets in the cytoplasm) encompassed by a large number of follicle cells (fc); FIG. 2: Late vitellogenic oocytes (lvo) with egg hull projections (arrow) showing ortho and metachromatic reaction of outer and inner layers. Scale bars Fig. 1 = 50 µm; Fig. 2 = 100 µm.



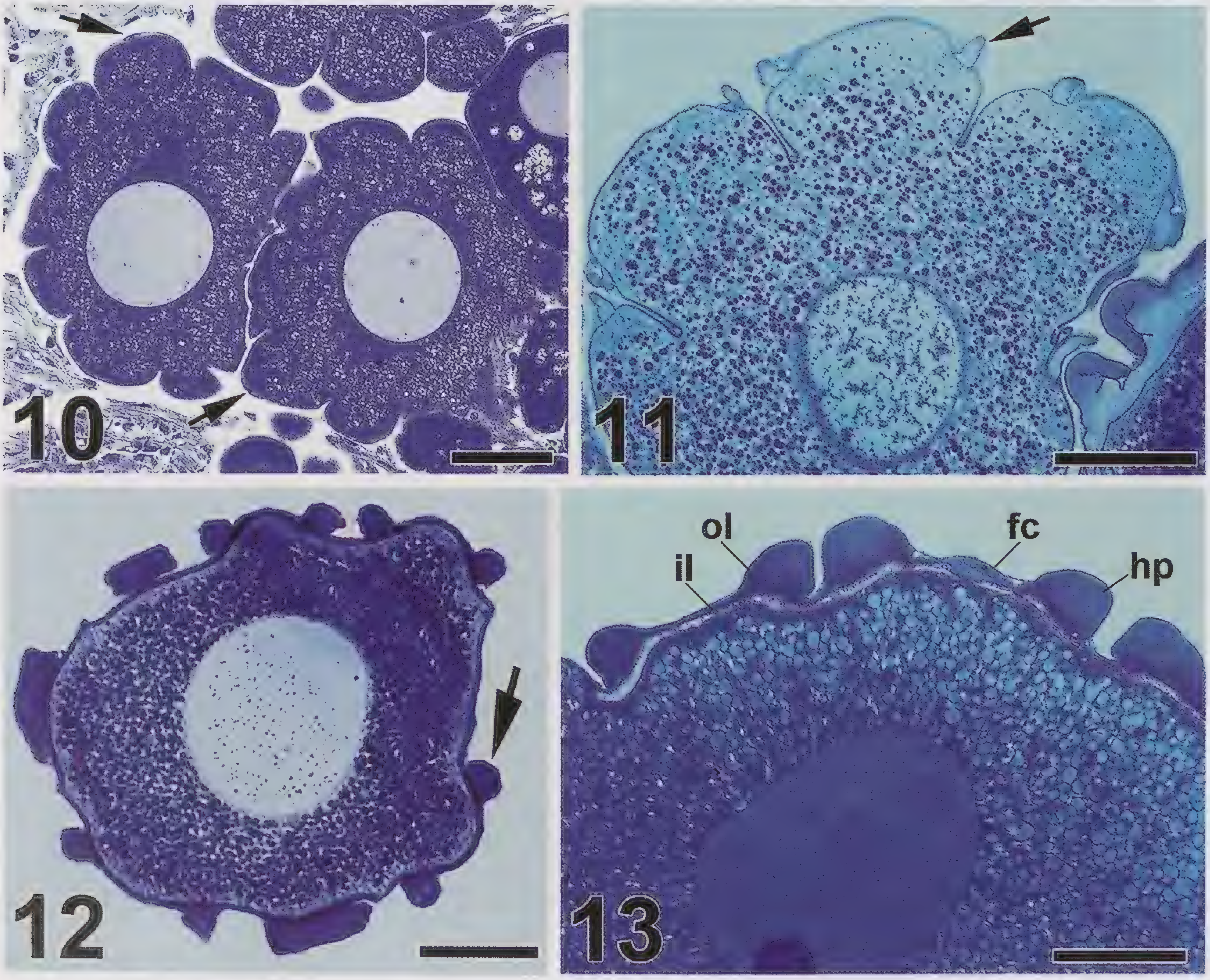
FIGS. 3–9. *Chaetopleura isabellei*. FIGS. 3–7. SEM photographs of egg hull ultrastructure; FIG. 3: General view of an egg; FIG. 4: Close-up of four egg hull spines; FIG. 5: Detail of the arrangement of leaflet-like thorns on the tip of a spine; FIG. 6: Lateral view of dome-shaped base of a spine with micropores (arrow); FIG. 7: Inner view of an egg hull showing the polygonal basal plates with micropores (arrow). FIGS. 8, 9: SEM of sperms. FIG. 8: Lateral view of a sperm showing nuclear filament (nf) and mitochondria (m) at the base of the nucleus; FIG. 9: A sperm showing the four laterally placed mitochondria (m). Scale bars Fig. 3 = 100 μm; Fig. 4 = 20 μm; Figs. 5, 6 = 5 μm; Fig. 7 = 10 μm; Figs. 8, 9 = 2 μm.

to about 240 µm). Egg hulls were sculpted with hollow projections also referred to as spines, about 50 µm length (Figs. 3, 4). Each projection extends from a dome-shaped hexagonal plate of about 20 µm diameter, which continues in a somewhat short, cylindrical column that ends in a club-shaped closed tip (Figs. 4, 6, 7). The tip is surrounded by four to six whorls of thorn-like projections; successive whorls of thorns were distributed following an alternate pattern (Fig. 5). At the inner and outer surface of the dome-shaped base of each projection, numerous open micropores were observed (Figs. 6, 7).

Sperm of *C. isabellei* showed a bullet-shaped nucleus (about 3 µm length), with an elongate nuclear extension of about 2 µm length (Fig. 8), and four mitochondria, somewhat laterally displaced, at the base of the nucleus (Fig. 9).

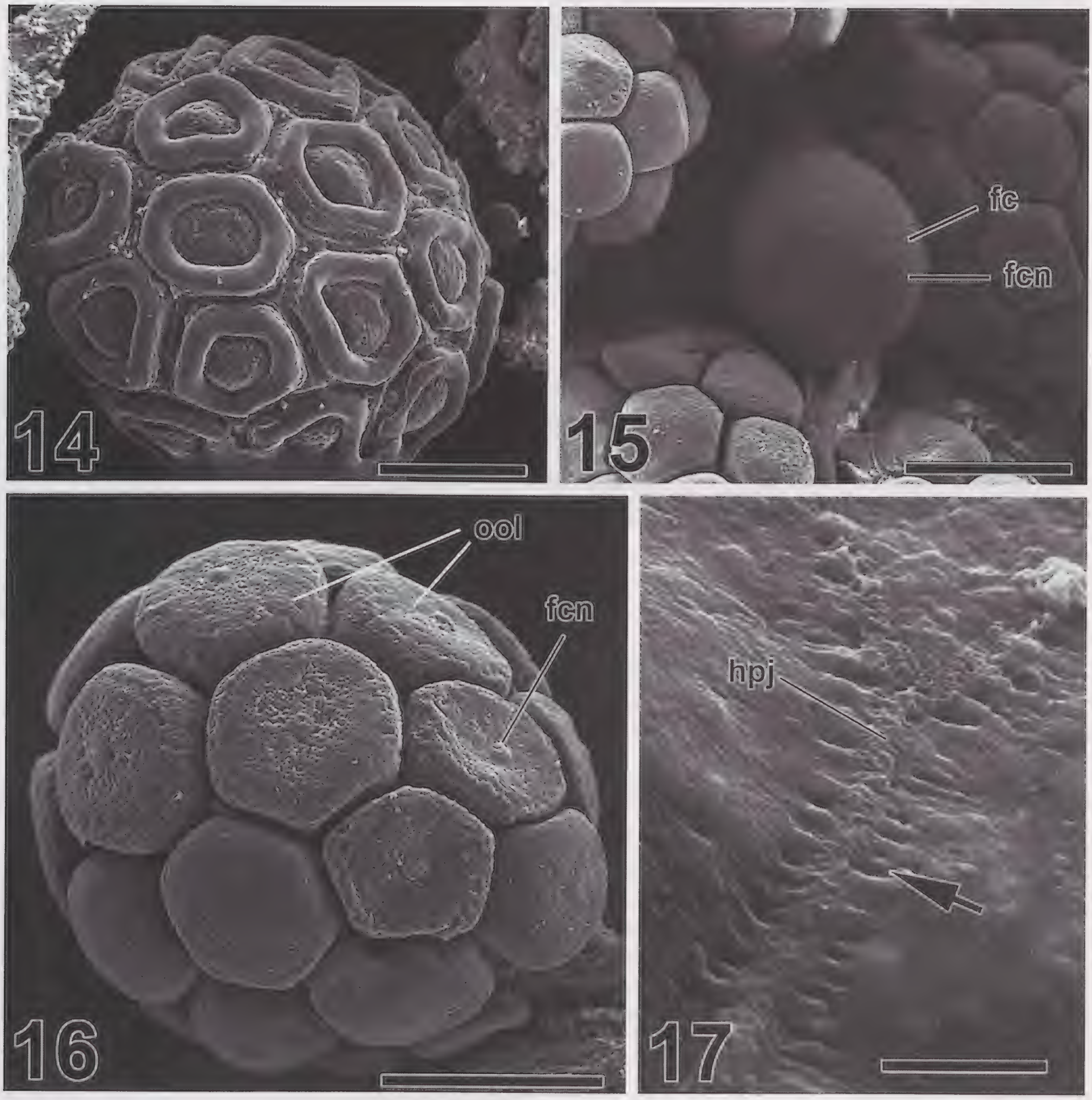
Plaxiphora aurata

Late previtellogenic or early vitellogenic oocytes of *Plaxiphora aurata* (Mopaliidae (?): Acanthochitonina) are encompassed by a reduced number of follicle cells (less than 10 per transverse section) (Fig. 10), each delimiting a polygonal (pentagonal or hexagonal) space on the surface of oocytes (Fig. 15). At the beginning or just after starting vitellogenesis, the ooplasm showed a series of lobules, which in transverse sections appear as deep incisions, determined by the infolding of the oocyte membrane (Figs. 10, 11, 16). The deepest part of each incision corresponded to the contact zone of two contiguous follicle cells (Fig. 10). As vitellogenesis progressed, the deposition of materials forming the egg hull begins and progressively the ooplasm lobules reduce in size, vanishing in late vitel-

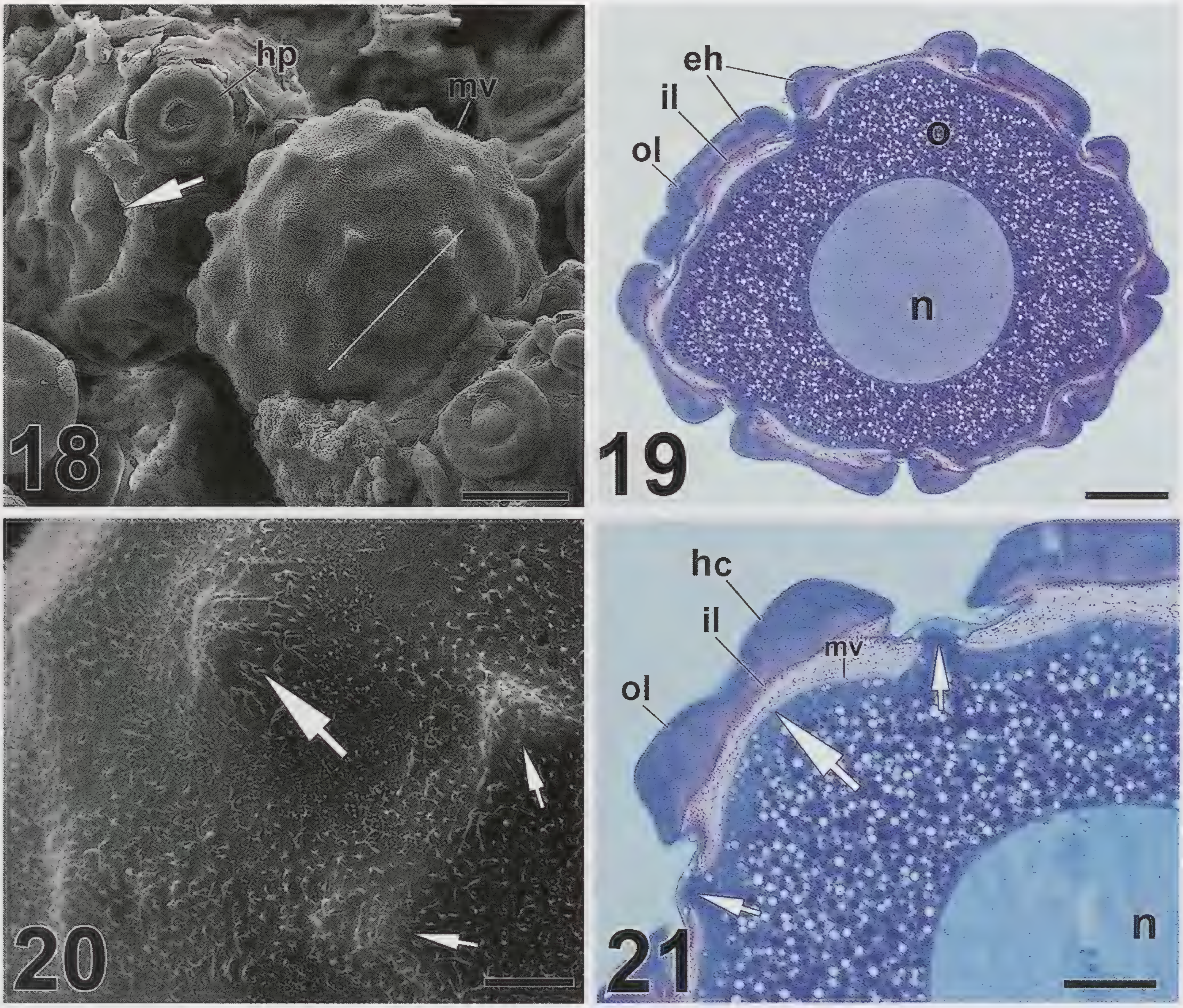


FIGS. 10–13. Oogenesis in *Plaxiphora aurata*. FIG. 10: A late previtellogenic oocyte showing the lobulations of the ooplasm, each one with an associated follicle cell (arrows); FIG. 11: An early vitellogenic oocyte at the initial phase of egg hull formation (arrow); FIG. 12: A mid vitellogenic oocyte with nearly inconspicuous ooplasm lobulations (arrow indicates egg hull); FIG. 13: Late vitellogenic oocyte with the hull in an advanced stage of development showing the ortho and metachromatic reactions of outer (ol) and inner (il) layers of hull wall, and a follicle cell (fc) still associated to a hull projection. Scale bars = 50 µm.

logenic or ripe oocytes (Figs. 11–13, 19). Egg hulls in *P. aurata* are composed of polygonal, usually pentagonal or hexagonal plates, 50 µm in diameter, with low areole-shaped projections; the somewhat inflated border of the areole surrounds a depressed area, slightly elevated in the centre by a very low cupule (Fig. 14). The egg membrane underneath the egg hull shows clearly demarcated polygonal areas, covered with relatively long, uniformly sparse microvilli that reproduce the pattern, and coincide with the polygonal bases of the egg hull projections (Figs. 18, 20). At each of these areas, the egg surface show a central bulge at the place formerly occupied, during early stages of hull formation, by the nucleus of the associated follicle cell (Figs. 13, 18, 20, 21), and corresponding to the slightly elevated centre of each hull cupule. Five or six peripheral points coincide with each of the angles of the polygonal base of adjacent hull cupules (Figs. 18, 21). Along the perimeter of each polygonal base of hull cupules, rows of somewhat large and regularly spaced open micropores (0.5–0.7 µm diameter) were present



FIGS. 14–17. *Plaxiphora aurata*. FIG. 14: General view of an egg; FIG. 15: Early vitellogenic oocyte (centre of image) showing polygonal shape of follicle cells (fc) with central nucleus (fcn); FIG. 16: SEM of an advanced vitellogenic oocyte showing the infoldings of the plasma membrane and ooplasm lobulations (ool) with centrally located nucleus of follicle cells (fcn); FIG. 17: Micropores (arrow) at the perimeter of contacting basal plates. Scale bars Figs. 14–16 = 50 µm; Fig. 17 = 5 µm.



FIGS. 18–21. *Plaxiphora aurata*. FIG. 18: SEM of two late vitellogenic oocytes, one (upper left corner) with the egg hull partialy removed (arrow) and another (centre) with egg hull completely removed, showing the cups of ooplasm, and plasma membrane with microvilli (white line indicate position of section showed in figure 21); FIG. 19: Transverse section of an egg with egg hull completely developed (eh. egg hull, hp. hull plate, mv. microvilli, n. nucleus, o. ooplasm); FIG. 20: Close-up of figure 18 showing the central bulge of ooplasm beneath a hull plate (large arrow) and minor bulgings (small arrows) coinciding with the hull plate periphery; FIG. 21: Transverse section of an egg showing central (large arrow) and peripheral (small arrows) bulgings of the ooplasm beneath a hull cupule (hc) (mv. microvilli; il. inner hull layer; ol. outer hull layer). Scale bars Figs. 18, 19 = 50 µm; Fig. 20 = 10 µm, Fig. 21 = 20 µm.

(Fig. 17). In transverse sections of fully developed egg hulls stained with tolouidine blue, two component layers were distinguished, the outer orthochromatic and the inner metachromatic, evidence of the probable presence of neutral and acid mucopolysaccharides (Figs. 13, 19, 21).

DISCUSSION

According to Buckland-Nicks (2008), *Chaetopleura apiculata* shows hollow complex branching spines, a morphology unknown from any other genera. Finding very similar spines in another member of *Chaetopleura* implies that such spines are characteristic of this genus and likely homologous. The egg hull of *Chaetopleura isabellei* and *C. apiculata* are similar to those of other members of Chitonina in the general morphology of the spines, extending from a relatively narrow, dome-shaped polygonal base and formed by a short, hollow column with a closed tip. Compared with *C. apiculata*, eggs of *C. isabellei* are smaller (about 20%), the spines longer and the tips are club shaped, bearing a quite peculiar array of thorn-like projections, arranged in several whorls. In *C. apiculata*, the spine branching is mainly on the proximal half instead of at the distal tip, as in *C. isabellei*. As well as in *C. apiculata* (Buckland-Nicks & Brothers, 2008), the projections of *C. isabellei* are hollow, and the hull is perforated at the base of each projection by minute open pores. Despite the lack of detail of Sirenko's (2006) drawing depicting a hull spine of *Chaetopleura angulata*, it looks similar to those of *C. isabellei* here described. The participation of the follicle cells during hull formation seems also similar to that described by Selwood (1970) in Chitonidae, a different family within Chitonina, specifically in *Sypharochiton septentriones* (Ashby, 1924), in which a large number of follicle cells encompasses each developing oocyte, and are progressively pushed apart from the egg membrane while the materials forming the hull are deposited. Even though the fertilization process was not studied here, it seems probable that the micropores in the base of the egg hull spines correspond to those described for *C. apiculata* as the sites for sperm penetration (Buckland-Nicks, 2008).

The general morphology of the egg hull of *Plaxiphora aurata* is similar to that sketched by Sirenko (1993, 2006) for *Plaxiphora kamehamehae* (Ferreira & Bertsch, 1979) and an unidentified *Plaxiphora* species. The reduced number and the peculiar behavior of follicle cells during oogenesis participating in the formation of several lobules of the ooplasm in *P. aurata* is similar to that described by Garnault (1888) and Richter (1986) in *Cyanoplax* (= *Lepidochitona*) *cinerea* (Linnaeus, 1767); however, the shape of the resulting egg hull in both species is quite different. Egg hull morphology of other species of the genus *Cyanoplax* (= *Lepidochitona*) have been studied in detail (Eernisse, 1988), but nothing is known on details of oogenesis in these species. The process of infolding of the oocyte membrane accompanied by follicle cells during early stage of oogenesis, has also been described by Anderson (1969) in *Mopalia muscosa* (Gould, 1846), Barnes (1972) in *Tonicella lineata* (W. Wood, 1815), Nimitz & Giese (1964) in *Katharina tunicata* (W. Wood, 1815), and Tucker (1960) in *Cryptochiton stelleri* (Middendorff, 1847). It is interesting to note that the peculiar behavior of the ooplasm-follicle cell complex during the early stages of oogenesis is only shared by other species of phylogenetically closely-related genera also included within the Chitonida suborder Acantochitonina (*sensu* Sirenko, 2006). This should be viewed as an additional character reinforcing the monophyly of the Acanthochitonina, relatively well supported by molecular information, morphology of egg hull, sperm morphology, and mode of fertilization (Okusu et al., 2003; Buckland-Nicks, 2008). Based on different sets of apomorphic characters, two groups among the Acanthochitonina have been recognized (Buckland-Nicks, 2008). The first has egg hulls with closed cupules and fertilization between cupules, represented by the condition reported in *Cyanoplax* and *Acanthochitona*. The second has, among other similarities, egg hulls with open cupules and fertilization inside cupules, and corresponds to that found in *Mopalia*, *Tonicella*, *Cryptochiton*, which were found to be closelly related by Kelly & Eernisse (2008). Because the first condition is probably more widespread within Acanthochitonina, it is probably that it is plesiomorphic within Acanthochitonina, and the second condition is derived for a group that includes *Mopalia*, *Tonicella*, and *Cryptochiton*. *Plaxiphora aurata* clearly falls within the first group. From the previous and based on the peculiarities of the egg hull morphology here described, and, considering that this is a significant character in chiton taxonomy (Eernisse, 1984; Sirenko, 1993; Buckland-Nicks, 2008), it seems more adequate to place *Plaxiphora* outside Mopaliidae. Vendrasco et al. (2008), studying the aesthete canal morphology in Mopaliidae, arrived at the same conclusion.

Buckland-Nicks & Schander (2008) pointed out the plesiomorphic condition of such features as the presence of pores in the polygonal bases of the hull and a series of membrane cups just beneath the spine bases, they found in a member of the Chitonina, *Stenosemus albus* (Linnaeus, 1767). This condition, in their opinion, is reminiscent of that observed previously by Buckland-Nicks & Hodgson (2000) in *Callochiton dentatus* (Spengler, 1797), suggesting the position of *S. albus* as basal to the Chitonina. Other Chitonina and Acanthochitonina have pores in the hull (e.g., *Chaetopleura apiculata*, *C. isabellei* and some species of *Chiton*) (Buckland-Nicks, 2008). However, all of them lack membrane cups beneath the spine bases, or at least they have not been reported yet. Interestingly, in *Plaxiphora aurata*, the egg membrane underneath the egg hull showed clearly demarcated areas, that reproduce the pattern, and coincide with the polygonal bases of the egg hull projections, resembling the condition described in *S. albus*. If these areas of the membrane egg in *P. aurata* correspond to the membrane cups described by Buckland-Nicks & Schander (2008), both supposed plesiomorphic characters would be present in *P. aurata* from Patagonia, a species clearly related, in general morphology and peculiar features of oogenesis, to Acanthochitonina. However, the membrane cups in *P. aurata* are slightly different and may not represent the same structure than in *S. albus* and *C. dentatus*.

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DESCRIPTION OF THE RADULA OF *BUCCINANOPS GLOBULOSUS* (NEOGASTROPODA, NASSARIIDAE) AND AN ASSESSMENT OF ITS VARIABILITY IN NORTHERN PATAGONIAN GULFS

María S. Avaca[1, 2*], Maite Narvarte[1, 3] & Pablo R. Martín[1, 4]

INTRODUCTION

Buccinanops globulosus (Kiener, 1834) is an endemic macrofaunal component of coastal waters of the southwestern Atlantic Ocean (34°–48°S). This species inhabits sandy or muddy patches in the low intertidal zone to 10 m depth, where it spends most of the time buried (Scarabino, 1977). Its diet is mainly composed by carcasses of dead crabs, *Cyrtograpsus angulatus* and *Neohelice granulata* (Daleo et al., 2005), or the clam *Amiantis purpurata* (Narvarte, 2006). It has a great capacity to detect and rapidly consume carrion, generally outcompeting other scavengers (Daleo et al., 2005).

This species shows great interpopulation variability in many life history traits, including growth rate, size and age at first maturity, and fecundity (Narvarte et al., 2008). Such variation has been attributed to the effect of different environmental conditions, such as food availability, thus being an expression of phenotypic plasticity. Development is direct; crawling juveniles emerge and live in the same area as adults, thus restricting dispersal capability (Penchaszadeh, 1971). Owing to this reproductive strategy and the reduced mobility of adults, *B. globulosus*, as other species of gastropods with similar life history, may form isolated enclaves and may adapt to local conditions (Johannesson & Johannesson, 1993; Johannesson et al., 1993; Rochette et al., 2003; Sokolova & Boulding, 2004).

The radula is the major feeding organ of most gastropods, its structure related to diets and feeding modes. Radular characteristics have been employed for taxonomic purposes, being considered specific to species (Fretter & Graham, 1984), and at the generic and supra-generic levels (Reid, 1996; Nishi & Kohn, 1999; Franklin et al., 2007). However, intraspecific variation has also been reported and linked to differences in diet, food availability or habitat, and between ontogenetic stages, sexes, or populations (Nybakken, 1990; Padilla, 1998; Maintenon, 2004; Onitsuka et al., 2004; Andrade & Solferini, 2006).

The radula has been described for only two out of the five to seven species currently recognized in the genus *Buccinanops* (Pastorino, 1993; Simone, 1996), and little is known about its intraspecific variability. In this work, we describe the radula of *B. globulosus* and asses its variability among and within three populations in northern Patagonia. Populations selected for this study differ in maximum size, growth rate, size and age at first maturity, and fecundity (Narvarte et al., 2008; Avaca et al., 2009). Taking into account these differences and their possible relationship with environmental conditions at each site, it is interesting to discern if those differences also include radular characteristics.

MATERIAL AND METHODS

Sampling sites were located in the north Patagonian gulfs San Matías (SMG, two sampling sites: Villarino Beach and San Antonio Bay) and Nuevo (NG, one sampling site: Nueva Bay) (Fig. 1).

San Matías Gulf is the largest north Patagonian gulf, with a surface area of approximately 20,000 km^2, while Nuevo Gulf is relatively small, having only 2,400 km^2 (Rivas, 1990). Villarino Beach (40°45'S, 64°30'W) is an exposed beach located in northern SMG. Tides have a semidiurnal regime, with mean and maximum amplitudes of 6.32 m and 8.76 m, respectively. The slope of the beach is slight, and at low tides a large area of tidal flats, approximately 450–

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[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
[2] Instituto de Biología Marina y Pesquera Almirante Storni, Av. Güemes 1030, 8520 San Antonio Oeste, Río Negro, Argentina
[3] Instituto Multidisciplinario de Investigación y Desarrollo de la Patagonia Norte (IDEPA), San Martín 247, 8520 San Antonio Oeste, Argentina
[4] Laboratorio de Ecología, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina
*Corresponding author: msavaca@yahoo.com.ar

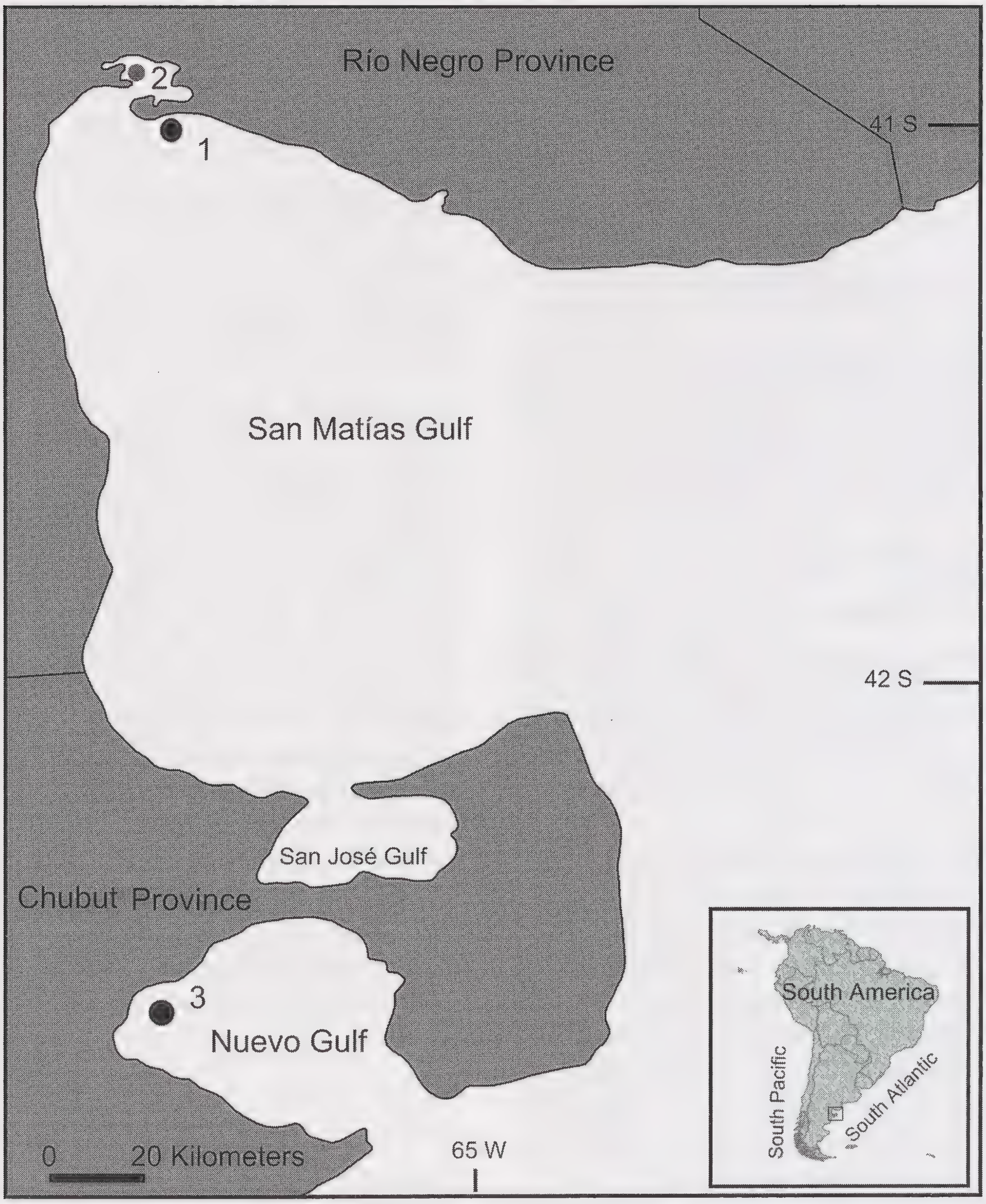


FIG. 1. Map showing sampling sites for *Buccinanops globulosus*; Villarino Beach (1), San Antonio Bay (2) and Nueva Bay (3).

600 m wide, is exposed (Morsan, 2003). The bottom is composed mainly of fine sand. At this site, *B. globulosus* occupies the intertidal and subtidal zone where the purple clam, *Amiantis purpurata*, is the main macrofaunal component (Morsan & Orensanz, 2004). San Antonio Bay (40°45'S, 64°56'W) is a salt marsh located in a semiclosed area in the northwest coast of SMG (Schnack et al., 1996). It has a semidiurnal tidal pattern, with a range variable from 6 m to 9 m and a maximum depth of 4 m at low tide. This area is highly influenced by local atmospheric conditions (Rivas, 1990, 1994). Small pebbles alternating with sand flats and large beds of the mussel *Brachydontes rodriguezii* constitute the main substrata.

Nueva Bay (42°45'S, 65°02'W) is located in the west of Nuevo Gulf near Puerto Madryn, one of the most urbanized coastal sites of Argentine Patagonia. Tides have a semidiurnal regime, with mean and maximum amplitudes of 3.66 m and 5.47 m, respectively. The slope of the beach is low, with an extensive intertidal zone of approximately 250 m wide. The bottom is formed by coarse sediments, mostly sand with sparse gravel and shell fragments, with outcrops of base rocks in some places (Solís, 1998).

Specimens of *B. globulosus* were collected during a two-year period (2006–2008). At diurnal tides, fish baits were employed to attract whelks during both low and high tides. Although whelks rapidly emerge from the sediment in response to food stimuli, bait was left for 20 min in order to ensure the attraction of individuals of all size classes present. Attracted whelks were collected (by snorkeling during high tides) and carried in plastic bags filled with sea water to the laboratory.

Once in laboratory, whelks were frozen in order to facilitate the dissection of radulae. Total shell length (TSL, parallel to the columelar axis) was measured using Vernier calipers (± 0.01 mm). Due to the occurrence of imposex in two of the studied populations, San Antonio Bay and Nueva Bay (Bigatti et al., 2009; Nudelman et al., in press), sex was determined on the basis of the presence of an albumen gland in females and by its absence and the presence of a penis in males.

Each individual was dissected when defrosted, and its radula was removed from the proboscis. Radulae were cleaned in 5% sodium hypochlorite in distilled water for 10 min, dried at 25°C and mounted in water on glass slides. The number of rows (NRO) per ribbon, the number of rachidian (NRC) and lateral cusps on each radula (NLC) were counted under a Nikon stereoscopic microscope. Total length of the radula ribbon (TRL) and the height of central rachidian cusp (HCC) of at least ten individuals per site were measured using a micrometric eyepiece.

Voucher specimens (mounted radulae) have been deposited in Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN) under the following collection numbers 37889, 37890 and 37891.

Scanning electron microscope (SEM) observations were made on four individuals from Villarino Beach and on three from the other two populations. For this purpose, 5 mm-long portions, were critical-point dried, mounted on stubs, gold coated, and observed with an EVO 40 SEM operated at accelerating voltage of 7 KV.

The description of the radula characters was based on Pastorino (1993) and Simone (1996); in our study, the central tooth is referred to as the rachidian tooth and cusps present in lateral teeth as inner, outer and middle. The counting of cusps was restricted to one row of the central part of the radula ribbon in order to avoid counting them on teeth worn or in a replacement process. The number of cusps on lateral teeth was recorded only from the right margin of each radula.

To test the relationship between TSL and morphometric variables of radula (TRL, NRO, NRC, HCC, NMC), Spearman's rank correlation analyses (Sokal & Rohlf, 1995) were performed for the pooled data and separately for each population. Differences among populations for all the radula variables were assessed using a non-parametric Kruskal-Wallis variance analysis, while intersex differences for the same set of variables were assessed by U test (Sokal & Rohlf, 1995). The α level of these tests was adjusted by Bonferroni correction.

Comparisons between sexes were assessed only with mature females and males, which were considered so if larger than the size at first maturity for females of each population (i.e., minimum shell length of a female carrying egg capsules: 14.5, 22.1 and 45.0 mm for San Antonio Bay, Nueva Bay, and Villarino Beach, respectively (Narvarte et al., 2008; Avaca et al., 2009). Owing to the intersexual differences in growth rate, male maturity may be attained at a smaller size than that of females, but no other external criterion of male maturity was available.

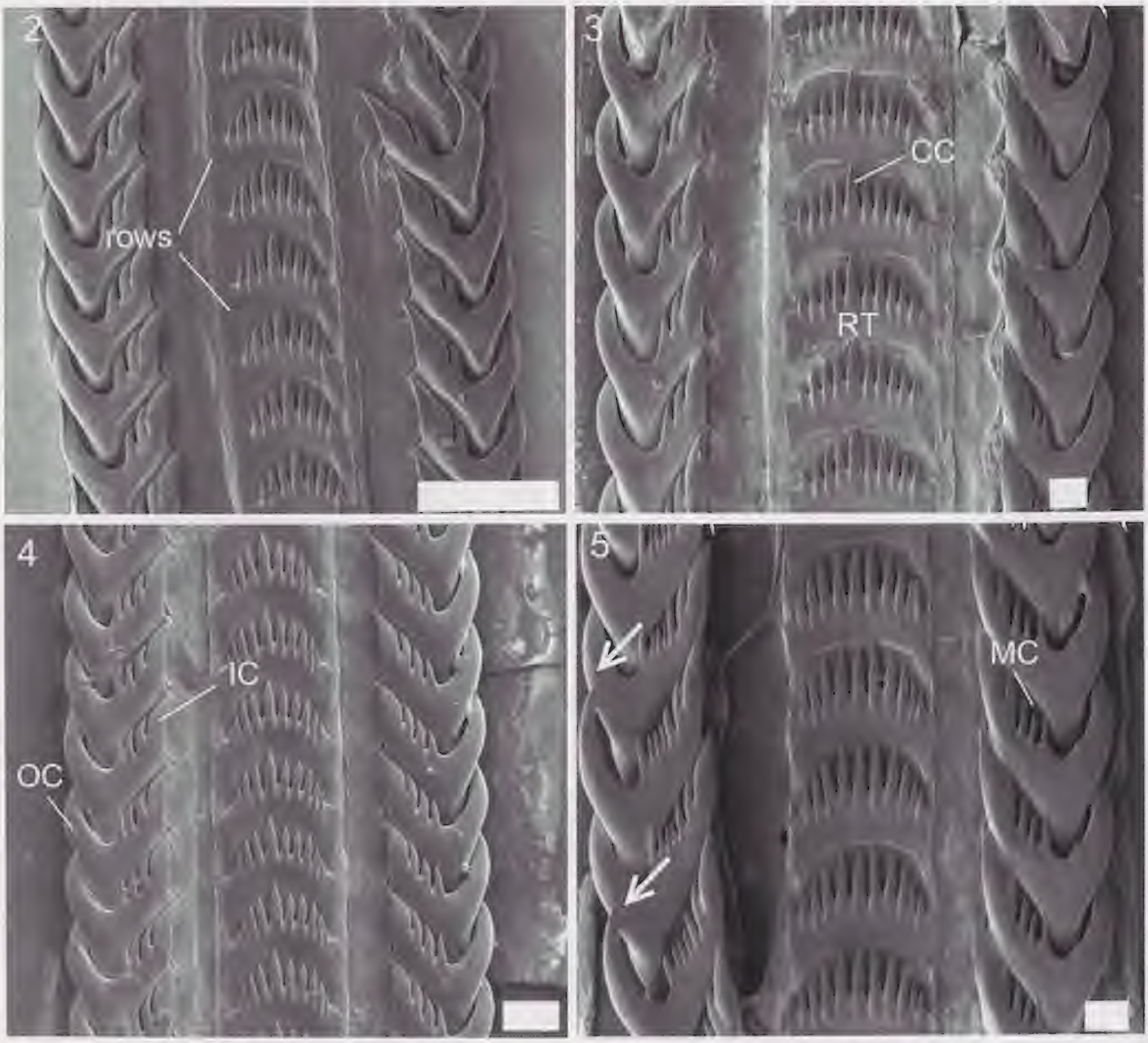
TABLE 1. Descriptive statistics for total shell length (TSL, mean ± standard deviation), number of rows (NRO, median), number of rachidian (NRC, median) and middle cusps (NMC, median) for the studied populations.

Population	TSL	NRO	NRC	NMC
San Antonio Bay	16.03 ± 3.54 (n = 77)	61	8	2
Range	8.35–24.82	37–77	6–10	1–3
Villarino Beach	35.65 ± 12.87 (n = 76)	70	9	2
Range	14.37–62.51	62–87	6–12	2–4
Nueva Bay	27.3 ± 8.7 (n = 81)	70.5	8	2
Range	13.47–46.77	54–83	6–10	2–4

RESULTS

Buccinanops globulosus has a rachiglossan radula, the number of transverse rows per ribbon varying between 37 and 87. The rachidian tooth was multicuspidate, with 6 to 12 well-spaced cusps that decrease in size from the central cusp outwards (Figs. 2–5). Generally, the number of cusps was the same to both sides of the central rachidian cusp. The rachidian teeth base was slightly concave. The lateral teeth have an inner cusp (sometimes bifid), 2–3 middle cusps and an outer cusp. The inner and outer cusps are curved towards rachidian tooth, the outer cusp being the largest. Differences in the number of middle cusps between right and left side were observed (Fig. 5). No differences in teeth shape among populations were observed.

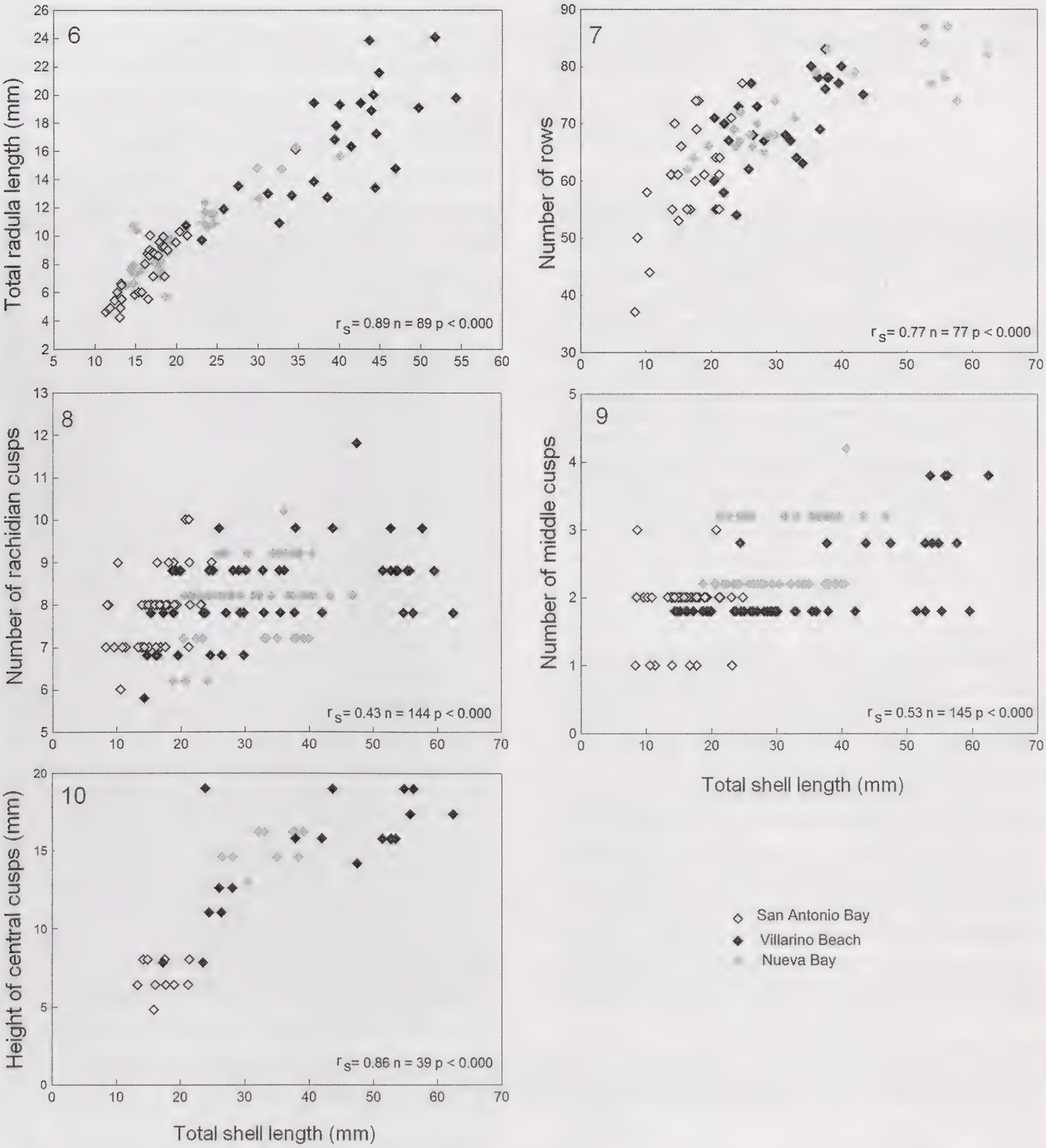
Specimens of *B. globulosus* varied markedly in size among populations (Kruskal - Wallis H (df = 2) = 114.01, $p < 0.001$, Table 1). Positive significant relationships between TSL and total radula length (TRL), number of rows (NRO), number of rachidian (NRC) and middle cusps (MMC), and central cusp height (HCC) were



FIGS. 2–5. Scanning electron micrographs (SEM) of radular teeth of *Buccinanops globulosus*. FIG. 2: Radula from San Antonio Bay, 8 rachidian cusps, 2 middle cusps; FIG. 3: Radula from Villarino Beach, 10 rachidian cusps, 3 middle cusps; FIG. 4: Radula from Nueva Bay, 10 rachidian cusps, 3 middle cusps; FIG. 5: Radula of an individual from Villarino Beach showing 5 middle cusps, bifid inner lateral cusps and the fusion of 2 lateral outer cusps (arrows). Scale bars: FIG. 2 = 200 µm; FIGS. 3–5 = 100 µm. CC = central cusp, IC = inner cusp, MD = middle cups, OC = outer cusp, RT = rachidian teeth.

found when the three populations were analyzed together (α global < 0.05) (Figs. 6–10); a steepest increase in the number of rows and in the length of the radula and central cusps was apparent for smaller whelks. Most of these relationships were also found to be significant within each population (Table 2); all the morphometrics variables of radula were positively and significantly correlated with shell size at Villarino Beach.

Intrapopulation variability was observed both in the number of rachidian cusps and in the number of middle cusps (Table 1). No sexual dimorphism was found in the radular variables,



FIGS. 6–10. Bivariate scatterplots for total shell length *versus* radular variables of *Buccinanops globulosus* in the studied populations. FIG. 6: Radula total length; FIG. 7: Number of rows per ribbon; FIG. 8: Number of rachidian cusps; FIG. 9: Number of middle cusps; FIG. 10: Height of central rachidian cusps. r_S: Spearman's rank correlation coefficient. In order to facilitate visualization ± 0.2 were added to the data of number of cusps for Nueva Bay and Villarino Beach, respectively.

TABLE 2. Summary of non parametric Spearman's rank correlation tests between total shell length and radula morphometric variables within each studied population. HCC: height of central rachidian cusps, NMC: number of middle cusps, NRC: number rachidian cusps, NRO: number of rows, TLR: total radula length, r_S: Spearman's rank correlation coefficient. **: $p < 0.001$, *: $p < 0.05$, ns: $p > 0.05$.

Population	TLR	NRO	NRC	NMC	HCC
San Antonio Bay	r_s = 0.87** n = 32	r_s = 0.60** n = 24	r_s = 0.51** n = 45	r_s = 0.49 ns n = 45	r_s = 0.05 ns n = 10
Villarino Beach	r_s = 0.75** n = 26	r_s = 0.86** n = 26	r_s = 0.45* n= 49	r_s = 0.59** n = 50	r_s = 0.66** n = 19
Nueva Bay	r_s = 0.85** n = 31	r_s = 0.68** n = 27	r_s = 0.26 ns n = 50	r_s = 0.32* n = 50	r_s = 0.47 ns n = 10

neither when pooling males and females from the three populations (NRO: H (df = 1) = 2.73, p = 0.08, n = 46; NRC: H (df = 1) = 0.41, p = 0.52, n = 85; NMC: H (df = 1) = 2.38, p = 0.12, n = 85 nor when comparing males and females within each population (U test p > 0.05 for each population and variable). Shell size differences were found between sexes within each studied population (U test, p < 0.001); intersexual comparisons could not be made within Villarino Beach population because only one mature male was contained in the sample.

DISCUSSION

The radula of *B. globulosus* has a morphological pattern similar to that of other species of *Buccinanops*, *B. cochlidium* and *B. moniliferus* (Pastorino, 1993; Simone, 1996; Table 3). This species has a rachiglossan radula with 6–12 rachidian cusps per row and lateral teeth with inner, middle and outer cusps. This type of radula is characteristic of non-active predators or scavengers (Castellanos, 1967). However, we have observed whelks inserting their proboscis between the open valves of healthy individuals of the purple clam, *Amiantis purpurata*, at one of the study sites (Villarino Beach), thus indicating that *B. globulosus* may also feed on live organisms. This is not surprising, because almost all predators are also facultative scavengers and vice versa (Kaiser et al., 2005).

The variation observed in the number of cusps (rachidian and laterals) and in height of central rachidian cusps among and within populations of *B. globulosus* do not involves the general radular morphology. These differences appear to be size related, as shown by the positive relationships found between these variables and total shell length. Several authors have reported increasing values in radula morphometric variables, such as length, width, and number of tooth rows with increasing total length, for instance in *Haliotis rubra* (Johnston et al., 2005) and in several muricids (Harding et al., 2008; Fujioka, 1985; Kool, 1993). However, despite size differences between sexes (Narvarte et al., 2008; this study), no sex-related differences were found in the radular variables. Similar results were found by Pastorino (1993) for the radula of *B. cochlidium*, while Simone (1996), despite noteworthy intersexual differences in size, did not mention radular differences between sexes in *B. moniliferus*.

Our findings suggest that feeding behavior of *B. globulosus* is similar across populations and that it remains constant during most of its

TABLE 3. Radular characters in *Buccinanops* species. NMC: number of middle cusps, NRC: number of rachidian cusps, TSL: total shell length (mm).

Species	TSL	NRC	NMC	References
B. moniliferus	up to 50	9–11	1–4	Pastorino, 1993
B. cochlidium	15.4–91.00	5–11	1–3	Simone, 1996; Pastorino, 1993
B. globulosus	8.35–62.51	6–12	1–4	This work

ontogeny. Field observations confirm that individuals of both sexes from a wide size range may feed on the same carcasses. Also a high degree of gregariousness in feeding activity, more than 30 individuals per carcass, was observed. This is common among scavengers, because carrion availability is ephemeral and umpredictable (Daleo et al., 2005), but it usually occurs in large amounts. For the smaller whelks studied, the variables related to the size of the radula and its teeth change faster relative to shell size than other size classes, suggesting a rapid growth of the radular system and proboscis. Perhaps this is related to the ontogenetic dietary changes (from detritivory to scavenging and predation) that have been reported for other nassariids (Tallmark, 1980). The newborn of *B. globulosus* do not feed on fish meat under laboratory conditions (pers. obs.).

Morphometric radular differences among the studied populations are clearly related to their variation in size. Differential growth rates observed in *B. globulosus* may be influenced by many factors, including food availability, presence of predators, and temperature. However, it seems that factors promoting size differences are not expressed in radula morphology. Narvarte et al. (2008) suggested that the high food availability observed at Villarino Beach and the low subtidal location of this population may explain the maximum sizes. At this site, the largest bed of the clam *Amiantis purpurata* along its entire distribution is found (Morsan, 2000). This bed could be acting as a carrion source for *B. globulosus* in two ways: directly by supplying of dead individuals and, indirectly, by carrion made by such predators as the crabs *Libinia spinosa* and *Ovalipes trimaculatus* and the gastropod *Odontocymbiola magellanica,* which feed on *A. purpurata* (Morsan, 2000; Narvarte et al., 2007). It is known that the availability of carrion for scavengers may be a limiting factor of growth, reproduction and eventually population size (Rochette et al., 1995; Morton & Chan, 1999; Rochette et al., 2001). Saunders et al. (2009) found that stunted and non-stunted populations of *Haliotis rubra* result mainly from a different food supply between sites.

We conclude that *B. globulosus* is homogeneous for radula characteristics; only size related differences were found among populations. This interpopulation uniformity in the radula supports the idea that the studied populations, despite the great variation in life history traits, would not represent different species, a possibility suggested in a previous work (Narvarte et al., 2008).

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A NEW INVASION INTO AN ANCIENT LAKE – THE INVASION HISTORY OF THE DREISSENID MUSSEL *MYTILOPSIS LEUCOPHAEATA* (CONRAD, 1831) AND ITS FIRST RECORD IN THE CASPIAN SEA

Katharina C. M. Heiler[1]*, Nahid Nahavandi[2] & Christian Albrecht[1]

INTRODUCTION

The Ponto-Caspian region, which includes the Black, Caspian and Azov Seas, plays a major role as both a source (Ketelaars, 2004; Reid & Orlova, 2002) and recipient (Grigorovich et al., 2003) for biological invasions. Invasions from the Ponto-Caspian region are known to have had a considerably effect on the Baltic Sea and the North American Great Lakes (Reid & Orlova, 2002; Ricciardi & MacIsaac, 2000). Approximately 70% of the species that invaded the North American Great Lakes between 1985 and 2000 were originally endemic for the Ponto-Caspian region (Reid & Orlova, 2002). For example, 16 Ponto-Caspian macroinvertebrate species were found in coastal waters of the Baltic Sea, of these nine species were Crustacea, three species Mollusca, and three species Oligochaeta (Bij de Vaate et al., 2002).

Grigorovich et al. (2003) recorded species invasions into the Caspian Sea and identified 36 non-indigenous species (NIS) of invertebrates; of these 12 species were Crustacea, eight species Trematoda and six species Mollusca. Most NIS were introduced from the Atlantic-Mediterranean region (i.e., Lusitanian region of Atlantic Ocean, Mediterranean and Black Seas) (Grigorovich et al., 2003).

The Caspian Sea is the largest continental water body in the world. It has a surface area of approximately 400,000 km^2, a maximum depth of 1,025 m, and contains brackish water with a volume of almost 79,000 km^3 (Dumont, 1998). It is structurally divided into three regions: Northern, Middle, and Southern Caspian with a salinity gradient ranging from 1–11 ppt in the northern to 12–13 ppt in the middle and southern part (Karpinsky, 1992). During summer the surface water temperature ranges from 24°C to 27°C and during winter from 0°C to 9°C (Reid & Orlova, 2002).

The Caspian Sea is believed to be the only extant lake formed from an ancient ocean (Riedel et al., 2006). The rate of endemism is high (Dumont, 1998; Kosarev & Yablonskaya, 1994). Endemic species in ancient lakes are often highly adapted to the conditions in their habitats and thus face higher risk when sudden changes in the ecosystems occur (Noges et al., 2008). Eutrophication and invasive species are two major threats to biodiversity in freshwater (Noges et al., 2008).

Often, when NIS arrive in the Caspian Sea, the invasion has already taken place in the Black Sea (de Mora & Turner, 2004). In 1952, the opening of the Volga-Don Canal facilitated the faunal exchange between the Caspian Sea and the neighboring Black Sea via the Don River and the Sea of Azov (Fig. 1). Shipping activities along the new invasion corridor even helped to increase the wave of introductions and became the major vector for NIS (Grigorovich et al., 2003). For example, the cirripedian crustaceans *Balanus improvisus* (Darwin, 1854) and *Amphibalanus eburneus* (Gould, 1841) established populations throughout the Caspian Sea after introduction from the Black Sea by ships via the Volga-Don Canal in the 1950s (Kosarev & Yablonskaya, 1994; Shiganova et al., 2005).

However, the Volga-Don Canal facilitates not only the immigration of exotic species to the Caspian Sea, but also the emigration of Caspian Sea species into other water bodies. One of the first mass invasions from the Ponto-Caspian region was caused by the biofouling zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Bij de Vaate et al., 2002). The economic and ecological damage caused by altering ecosystems and displacing other species have been immense (Connelly et al., 2007; Karatayev et al., 1997). Similarly, the quagga mussel, *Dreissena rostriformis bugensis* Andrusov,

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[1]Department of Animal Ecology & Systematics, Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32 (IFZ), 35392 Giessen, Germany

[2]Unit of Evolutionary Biology & Systematic Zoology, Department of Biochemistry & Biology, University of Potsdam, Karl-Liebknecht-Str. 24–25, 14476 Potsdam, Germany

*Corresponding author: katharinaheiler@gmx.de

TABLE 1. Invasion history of *Mytilopsis leucophaeata* (Conrad, 1831) in Europe.

Year of first record	Location	Source
1835	Antwerp, Belgium	Boettger (1928)
1895	Amstel River, The Netherlands	Maitland (1897)
1872	Northern France	Guerné (1873)
1928	Kiel Canal, Germany	Boettger (1932)
<1939	Kaliningrad Area, Russia	Zilch & Jaeckel (1962)
1993	Guadalquivir River, Spain	Escot et al. (2003)
1996	Cardiff Docks, South Wales, United Kingdom	Oliver et al. (1998)
1999	Thames River Estuary, E Coast, United Kingdom	Bamber & Taylor (2002)
2000	Warnow River, near Rostock, Germany	Darr & Zettler (2000)
2002	Black Sea Basin, Dniester Liman, Ukraine	Therriault et al. (2004)
2002	Canal Maritime, d'Aigues-Mortes, France	Girardi (2003)
2003	Loviisa Archipelago, Gulf of Finland	Laine et al. (2006)
2009	Southern Bug, near Mykolajiv, Ukraine	This study
2009	Caspian Sea, Iran	This study

1897 (other authors have given it species rank and treated it as *Dreissena bugensis*) from the Black Sea basin extended its range throughout wide parts of North America and more recently in western Europe (Martens et al., 2007; Molloy et al., 2007; Orlova et al., 2005).

Our study focuses on invasion dynamics of another dreissenid, Conrad's false mussel, *Mytilopsis leucophaeata* (Conrad, 1831), (Bivalvia, Dreissenidae), which has moved into the Caspian via one or several routes. *Mytilopsis leucophaeata* is native along the southern coast of North America (southern USA to Tampico, Mexico) (Marelli & Gray, 1983), and with past known invasion into wide parts of North America and Western Europe, particularly the North Sea coast and several inland waters such as the Kiel Canal and Rhine

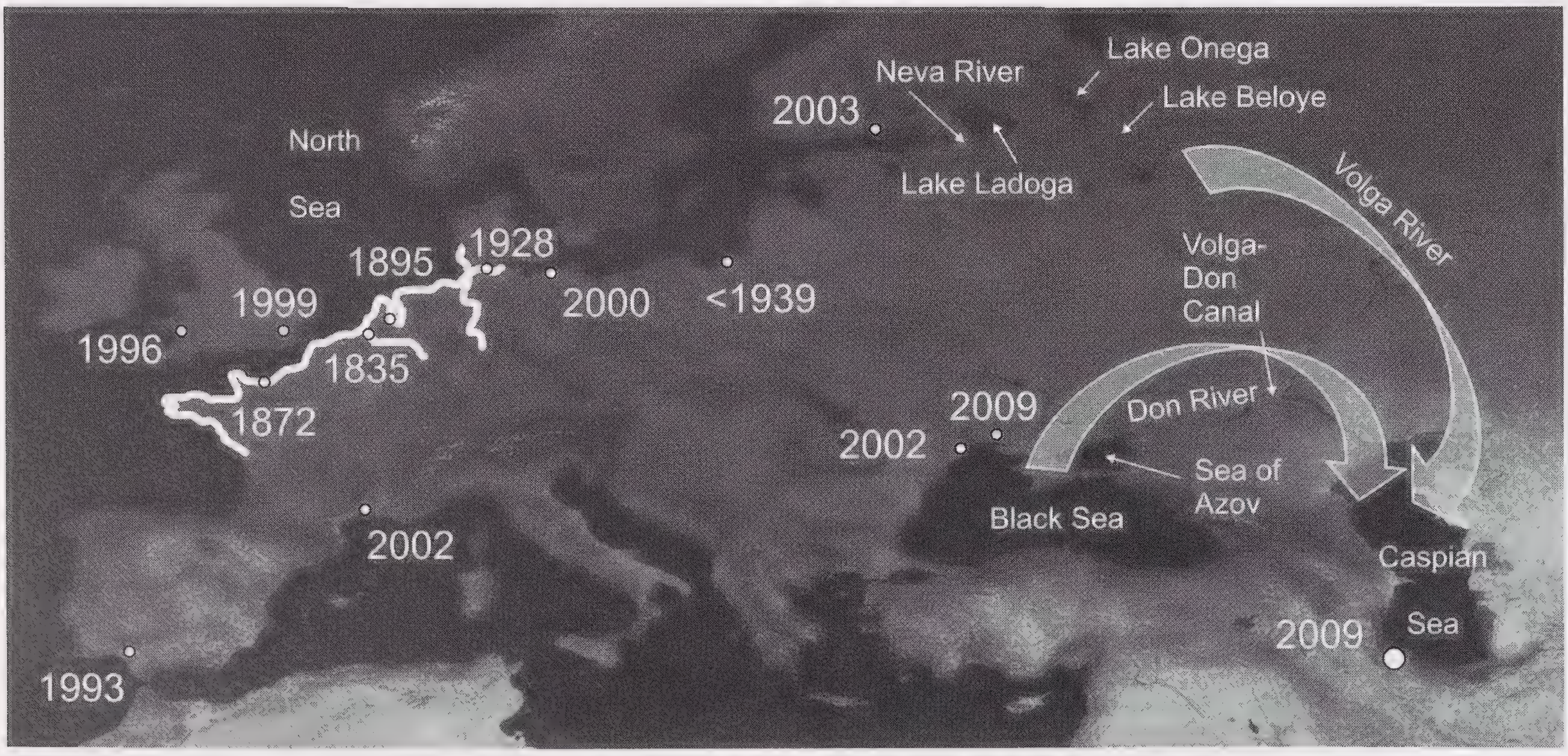

FIG. 1. Distribution of *Mytilopsis leucophaeata* (Conrad, 1831) (white line) and selected first records in Eurasia. The arrows indicate possible invasion routes into the Caspian Sea.

TABLE 2. Localities of sequenced individuals of *Mytilopsis leucophaeata* (Conrad, 1831), coordinates, and date of collection.

Location	Isolate/GenBank Accession #	Latitude (N) Longitude (E)	Date of Collection	Source
Belgium, Antwerp	5248/HM100251 5247/EF414477 11574/HM100252	51°21'34.99" 4°17'29.98"	19 Jan 2006	This study/ Albrecht et al. (2007)
Germany, Kiel Canal, near Baltic Sea	9984/HM100253	54°21'46.39" 9°49'09.68"	21 Oct 2008	This study
Germany, Kiel Canal, Brunsbüttel, near North Sea	9989/HM100254	53°54'36.77" 9°10'43.23"	20 Oct 2008	This study
Ukraine, Southern Bug, near Mykolajiv	12396/HM100255	46°58'49.26" 31°58'04.04"	3 Jul 2009	This study
Iran, Caspian Sea, Bandar Anzali	11313/HM100256 11314/HM100257 11317/HM100258 11572/HM100259 11573/HM100260 11788/HM100261 11794/HM100262	37°28'54.97" 49°27'37.01"	28 Apr 2009	This study
North America, Hudson River near Piermont, New York	U47649	n.a.	n.a.	Baldwin et al. (1996)

River and Weser River (Boettger, 1932; Jungbluth, 1996; Girardi, 2003; Therriault et al., 2004) (summarized in Fig. 1, Table 1). Like *D. polymorpha* and *D. r. bugensis*, *M. leucophaeata* has become a serious biofouling species in industrial pipe systems (Jenner et al., 1998; Rajagopal et al., 2002, 2005; Verween et al., 2005). It also has ecosystem engineering impacts similar to those of the zebra mussel (Pathy & Mackie, 1993; Sousa et al., 2009). Free-living planktonic larvae and high fecundity of dreissenids in general facilitates the transfer into new habitats and fast colonization (Bij de Vaate et al., 2002).

In 2002, it entered the Black Sea basin (Dniester Liman, Ukraine) (Therriault et al., 2004) and in 2003 the Loviisa archipelago, Gulf of Finland (Laine et al., 2006). These localities represent the most eastwards occurrences. In the Caspian Sea, there have been no records of this brackish water mussel until now.

MATERIAL AND METHODS

In April 2009, sampling to collect dreissenids took place in the southern part of the Caspian Sea from piers at a bridge in Bandar Anzali, Iran (Table 2) by A. R. Mirzajani and S. Rahim from the Inland Waters Aquaculture Institute. The bridge is located between brackish (Caspian Sea) and freshwater environments (mouth of the Sefid River) where many cargo boats and ships offload ballast water. The sampling was done by hand or with a dip net down to approximately 1 m depth. Specimens of bivalves and associated fauna were stored in 96% ethanol for further morphological and molecular examination. DNA, shell and soft tissue vouchers have been archived in the collection of the Department of Animal Ecology & Systematics at the Justus Liebig University of Giessen (UGSB). DNA from seven bivalves was isolated using protocols of Winnepenninckx et al. (1998) and Wilke et al. (2006). For amplification, the mitochondrial cytochrome oxidase *c* subunit I (COI) gene with a target length of 655 bp was used (Folmer et al., 1994). Primers were utilized for amplification and sequences were produced with a LI-COR (Lincoln, Nebraska) Long ReadIT 4200 sequencer and the Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech, Piscataway, New Jersey). Sequences were compared with available sequences from NCBI GenBank and with sequences from specimens sampled in 2006, 2008 and 2009 from the invaded areas in Belgium and Germany and the Ukraine (Table 2). All sequences have been sent to NCBI GenBank (Table 2: GenBank accession numbers).

For morphological determination, the shell was opened to examine the internal structure. The shell length of 60 individuals was measured with a calliper and the fouling rate (i.e. number of balanid specimens per shell) was calculated.

RESULTS

During the sampling in spring 2009 in the Southern Caspian, several hundred dreissenid specimens were found with encrusting cirripedian crustaceans. The estimated biomass of bivalves and balanids was approximately 3 kg per m^2, while the abundance of individual balanids exceeded number of the bivalves (73% balanids and 27% bivalves). The water temperature was 18°C, the average pH value in this area in spring was 7.9, and the average of the dissolved oxygen in spring was 8.9 mg/l. The shell morphological analysis of several mussel specimens from the Caspian Sea showed the existence of an apophysis on their myophore plate, which is present in *Mytilopsis leucophaeata* (Fig. 2), but absent in quagga or zebra mussels (Pathy & Mackie, 1993).

The COI sequences obtained confirmed the determination of this species as *M. leucophaeata*. The comparison of the COI sequence (528 bp) of seven individuals with *M. leucophaeata* from Germany (Kiel Canal), Belgium (Antwerp), Ukraine (Southern Bug), and North America (Savannah River, Georgia and Hudson River near Piermont, New York (GenBank: U47649)) (Table 2) revealed the presence of a single identical haplotype at all sites mentioned.

Shells sizes ranged from 5.5–22 mm (average 13.5 mm, n = 60). Thirteen of 60 specimens of *M. leucophaeata* examined were overgrown with *Amphibalanus eburneus* (Cirripedia). A maximum of five specimens of *A. eburneus* were found on one shell. Only mussels larger than 12.8 mm were overgrown.

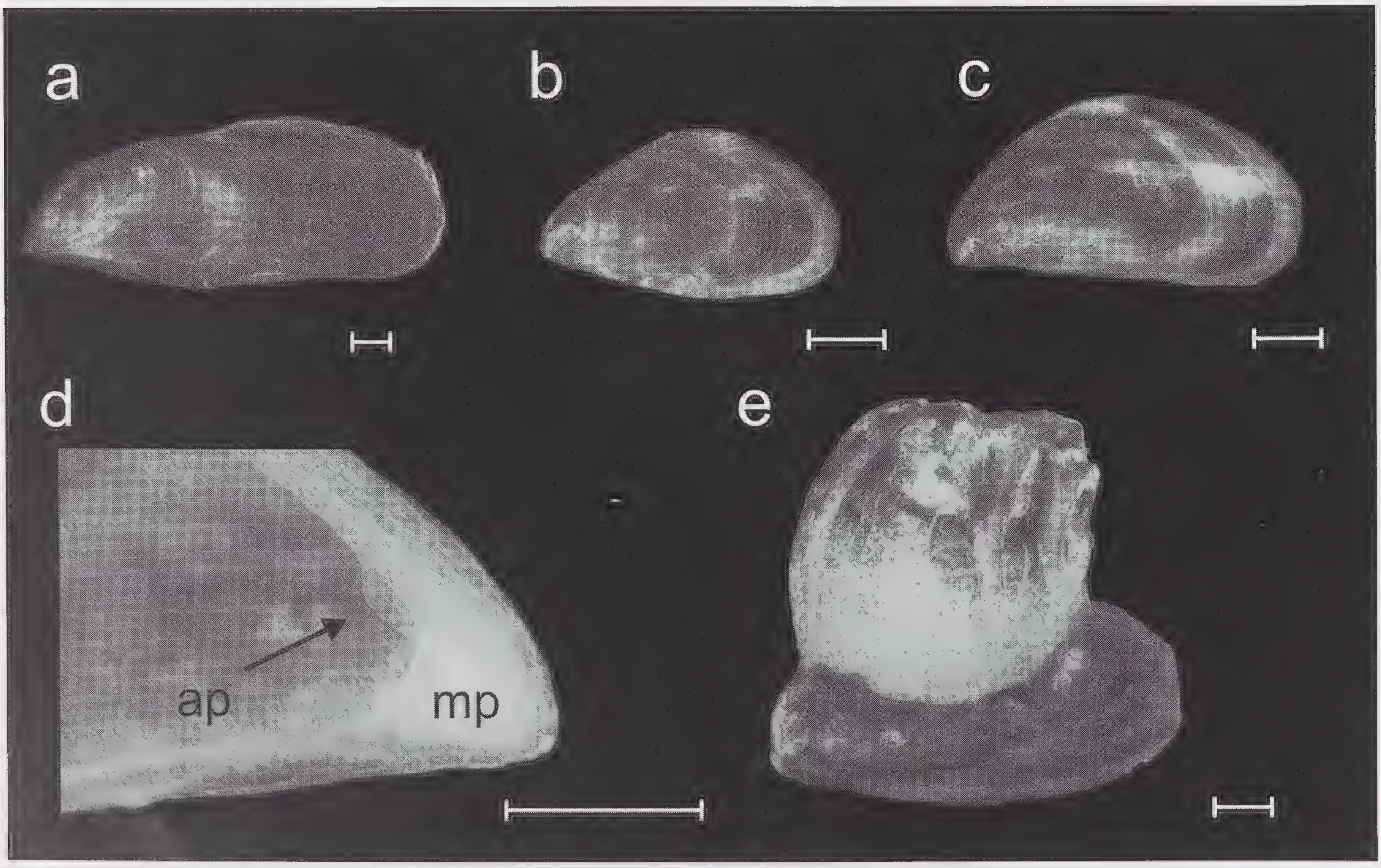

FIG. 2. Plate with three individuals of *Mytilopsis leucophaeata* (Conrad, 1831) from the Caspian Sea (11313 (a), 11573 (b), 11314 (c)), inner shell showing the apophysis (ap) of the myophoreplate (mp) (d) and *M. leucophaeata* overgrown with an individual of *Amphibalanus eburneus* (Gould, 1841) (e). Scale bar = 2 mm respectively.

DISCUSSION

Mytilopsis in the Caspian Sea

The population of *Mytilopsis leucophaeata* found in the Caspian Sea contained adult individuals with a maximum shell length of 22 mm. Verween et al. (2006) found the same maximum shell length in populations of *M. leucophaeata* in Belgium. Based on size, they calculated a longevity of greater than five years for this species. Thus, we assume that the larger individuals found in the Caspian Sea are approximately five years old and thus invaded the lake probably not later than 2004. Given the high densities we found and the fact that this population is at the far opposite end of the sea from the supposed entry point (Volga River Liman), it is likely that the first individuals might have invaded the Caspian Sea earlier.

The family Dreissenidae has been proposed to have originated in the Eocene, with a major radiation occurring in the Parathethyan Basin (today Black Sea, Caspian Sea, and Aral Sea) from the Late Miocene to the Pliocene (Starobogatov, 1994). While some species within this family are highly endemic, others have recently undergone a global redistribution, as summarized in Orlova et al. (2005). Transfer via shipping activities by means of transport as larvae in ballast water or as adults attached to ships' hulls with byssal treads seems to be responsible for the introduction of populations of *M. leucophaeata* from North America into Europe (Bamber & Taylor, 2002; Oliver et al., 1998) and was also presumed to be the case for the Black Sea (Therriault et al., 2004).

Canal construction, such as the Volga River system connecting the Caspian and Black Seas and the Baltic Sea, can serve as new invasion corridors for NIS overcoming physiological barriers (Reid & Orlova, 2002). The deletion of such barriers can have great impact on the endemic taxa by allowing the transfer of NIS between formerly isolated ecosystems (Therriault et al., 2004). This contact may cause competition between natives and endemics which may even lead to exclusion of the less competitive species (Albrecht et al., 2009).

The Caspian Sea mollusk fauna is mainly endemic and numerically dominated by cardiid and dreissenid bivalves and hydrobioid snails. Although the taxonomy of the Caspian Sea mollusk fauna is still not satisfactorily resolved (Wesselingh, 2007), several dreissenid taxa have been recognized. While Rosenberg & Ludyanskiy (1994) distinguish four dreissenid taxa in the Caspian Sea on the basis of morphology and salinity tolerance: *D. polymorpha*; *D. rostriformis* Deshayes, 1838; *Dreissena elata* Andrusov, 1897; and *Dreissena caspia* Eichwald, 1855, other authors split *D. polymorpha* and *D. rostriformis* into several subspecies based on differences in depth at which they occur (reviewed in Rosenberg & Ludyanskiy, 1994). The invasive dreissenid *D. r. bugensis* has recently invaded the northern Caspian Sea where the Volga River dilutes the salinity to 2 to 3 ppt (Orlova et al., 2004). While *D. r. bugensis* has an upper salinity tolerance limit of 7 ppt (Oliver et al., 1998), *M. leucophaeata* is more euryhaline, being able to survive in salinity ranging from 0.1 to 31 ppt (Verween et al., 2007). In its native region, *M. leucophaeata* is present in warm, temperate waters (Marelli & Gray, 1983), but this species may occur also in water bodies with much lower temperatures (e.g. in the Kiel Canal in Germany). However, these permanent populations of *M. leucophaeata* in Europe remain rare due to variations in salinity or too low temperatures during winter – for example, for the UK (Eno et al., 1997), for parts of the coastal region in Europe (Wolff, 2005), for the Kaliningrad area (Darr & Zettler, 2000), and for Weser River (Bäthe, 1996). Thus, the southern Caspian Sea might much better comply with the requirements of this species (12–13 ppt and 9°C in winter). Given this fact, we suppose that *M. leucophaeata* has the ability not only to establish permanent populations but also invade further parts of the Caspian Sea or might already be present there. During the sampling no other dreissenid specimens were found, indicating that in this part of the Caspian Sea *M. leucophaeata* exceeded far the numbers of endemic dreissenids and is the dominant species.

Invasion Route

The broad ecological tolerance of *Mytilopsis leucophaeata* led Therriault et al. (2004) to conclude that *M. leucophaeata* has the ability to spread throughout the Ponto-Caspian region. Although it is possible that in exceptional cases dispersal events of aquatic mollusks can occur over land via bird migration (e.g., Haase et al., 2010), dispersal via waterways is more likely. Two main invasion corridors are suggested to have been the routes for the invasion in the Caspian Sea: as this species was already present in the Black Sea basin in 2002, this bivalve may have been transported by ships via the Volga-Don Canal (Fig. 1). However, as established populations of *M. leucophaeata* in the Gulf of Finland were already recorded in 2003 (Laine et al., 2006), it could be also possible that this bivalve arrived in the Caspian Sea via the Volga River (Fig. 1). This invasion corridor includes long-distance larvae dispersal via the Baltic Sea, Neva River, Lake Ladoga, Lake Onega, Lake Beloye and Volga River (Fig. 1). This invasion corridor is one of the three invasion routes that Bij de Vaate et al. (2002) hypothesized for NIS emigrating out of the Ponto-Caspian region. However, it is also conceivable that NIS may enter the Ponto-Caspian region using these corridors. To clarify the question of the invasion route satisfactory, molecular studies including a more variable marker should be conducted.

The COI-gene has been shown to be very informative for population genetics in Balkan endemic dreissenids *D. blanci* Westerlund, 1890 and *D. presbensis* Kobelt, 1915, which are characterized by a high degree of variability (Wilke et al., submitted). However, in the invasive dreissenids *D. polymorpha* and *D. r. bugensis*, the invading populations show a low genetic variability in COI, presumably resulting from bottlenecks during invasion (May et al., 2006; Albrecht et al., in prep.). Despite the lack of genetic differentiation in invading dreissenids, founding populations seem to be very successful. While the low genetic differentiation among populations in the invaded range of *M. leucophaeata* is thus not surprising, it would be interesting to examine the genetic structure in their native region.

Conservation Issues

The biodiversity of the world´s ancient lakes is threatened by a variety of anthropogenic factors: introduction of invasive species, pollution and fishing activities are only some of the reasons why aquatic ecosystems have rapidly changed and extinction rates have increased dramatically (Cohen, 1994). Caspian biodiversity is threatened by two major kinds of human activities: (1) shipping transfer and connection of previously separated water systems, which has introduced NIS, and, (2) impairment in the water quality caused, for example, by oil exploitation and pollution (Stanners & Bourdeau, 1995). These dual threats work synergistically and may amplify impacts on the native fauna.

The Middle and Southern Caspian are particularly at risk, where decreasing species diversity of phytoplankton, zooplankton and macrobenthos has been reported (summarized in Stanners & Bourdeau, 1995). Other authors have already presumed the extinction of *Dreissena caspia* and *D. elata* following the invasion of *Mytilaster lineatus* (Gmelin, 1791) (Rosenberg & Ludyanskiy, 1994) and suggested that the invasion of *M. leucophaeata* may herald a further loss of native bivalves in the Black Sea (Therriault et al., 2004).

Because of its demonstrated high colonization success, ecosystem engineering impacts and tendency to dominate the fauna, this new invasion of *M. leucophaeata* is likely to pose a serious further threat to the endemic dreissenid fauna in the Caspian Sea.

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SIZE AND AGE AT FIRST SEXUAL MATURITY OF THE EDIBLE GIANT SNAIL *ADELOMELON BECKII* (NEOGASTROPODA: VOLUTIDAE) FROM MAR DEL PLATA, ARGENTINA

Florencia Arrighetti[1*] & Pablo E. Penchaszadeh[1, 2]

INTRODUCTION

The giant volutid *Adelomelon beckii* (Broderip, 1836) inhabits coastal areas of the western South Atlantic and is distributed from Espíritu Santo, Brazil, to Tierra del Fuego, Argentina, on sandy bottoms between 40 and 70 m (Weaver & Dupont, 1970). This species is one of the top benthic predators found within the studied area (Arrighetti, 2009) and has low population densities (Carranza et al., 2008). The Argentinean populations used to be harvested as part of the bycatch by fishing trawlers, but recently this species has been used for consumption and its large, attractive shell (up to 40 cm SL) sold as an ornament. Despite its impressive size and its increasing significance as a fishery resource, surprisingly little is known about the biology of *A. beckii* and its role in the ecosystem, except for some aspects of reproduction (Giménez et al., 2009; Arrighetti, 2009; Arrighetti & Giménez, 2010). Sexes are separate, with males having copulatory organs and the females laying egg capsules; each capsule contains 7–9 embryos and is attached to an empty bivalve shell (Penchaszadeh et al., 1999). *Adelomelon beckii* off Mar del Plata reproduces in spring and autumn (Arrighetti & Penchaszadeh, 2010) and is one of the species of gastropods with highest longevity thus far studied, reaching 29 years old (Arrighetti, 2009).

The objective of this study is to describe size and age at first sexual maturity of *Adelomelon beckii* in the fishing area off Mar del Plata using gonadal maturation studied by histology. These results will then be used to determine minimum catching size in order to establish a future management of this resource.

MATERIAL AND METHODS

Samples were collected monthly during two consecutive reproductive periods: from September 2004 to April 2005 (season 2004) and from August 2005 to April 2006 (season 2005) (Arrighetti & Penchaszadeh, 2010), so all mature specimens had mature gametes. The study was carried out by bottom trawling in the Mar del Plata area (38°20'S, 57°37'W) at a depth of 40–70 m. A total of 8–20 snails per month, ranging from 16 to 39 cm total shell length (SL) were studied. In the laboratory, each snail shell length was measured with a vernier caliper to the nearest 0.1 cm, and shell free wet mass (SFWM) was weighed to the nearest 1 g. Sex was identified by the presence or absence of a penis. The testis and the ovary in *Adelomelon beckii* cannot be clearly separated from the digestive gland, so no gonadosomatic index could be calculated. Sexual maturity was established using histological criteria. For both sexes, after removal from the shell, a small piece of the gonad and seminal vesicle was removed and fixed in Bouin's solution for 3 h at room temperature and subsequently transferred to 70% alcohol for storage. Tissues were dehydrated using an ascending series of ethanol dilutions and then embedded in resin (Leica Historesin). Sections were cut at 5 μm thickness with an electronic microtome (Leica), stained with hematoxylin-eosin, and observed under a light microscope (Zeiss Axioimage Z. 1). Females were considered mature when oocytes undergoing vitellogenesis were present and males when spermatozoa were present in the spermatic tubules and in the seminal vesicle.

Statistical difference between SL distributions between the two reproductive seasons was

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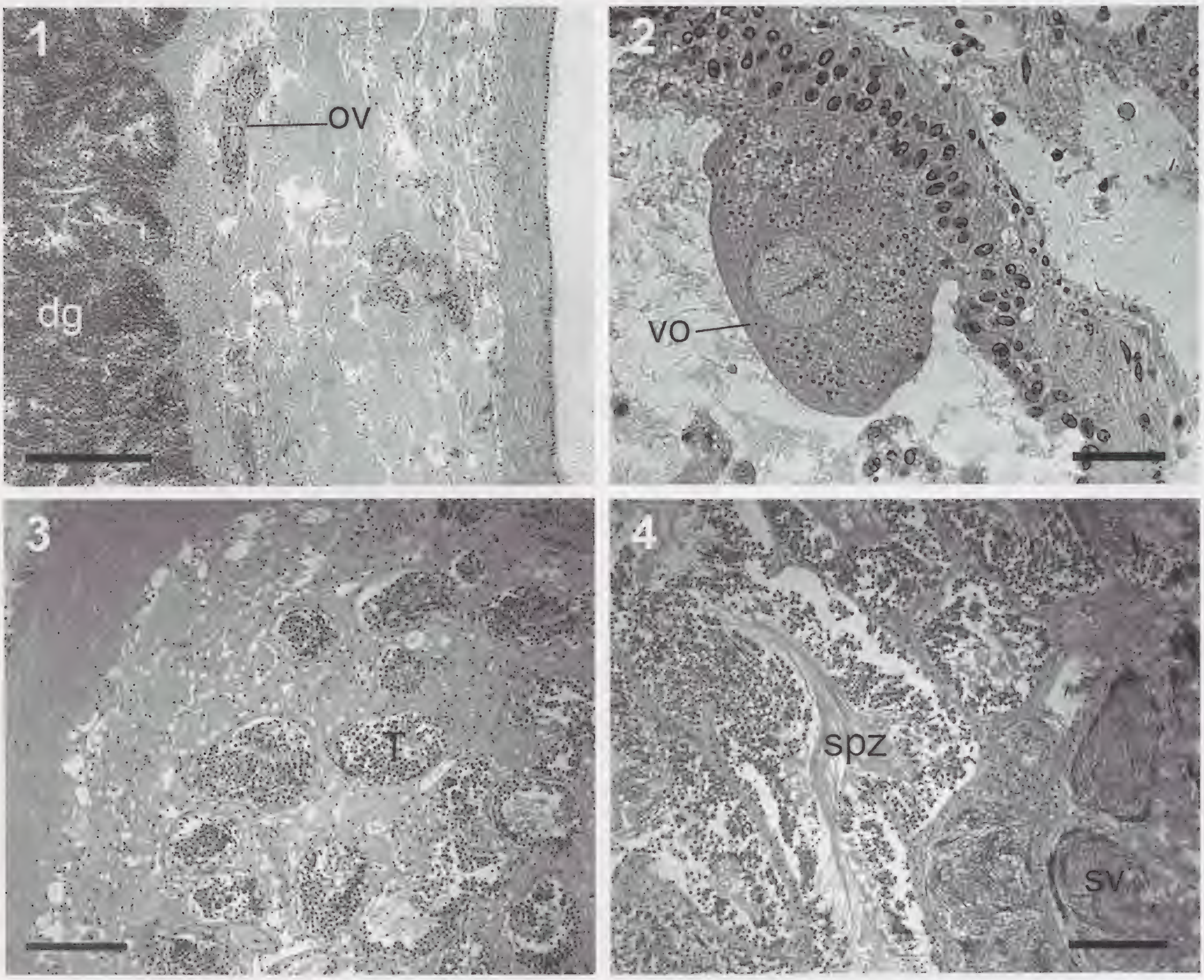
[1]CONICET [Consejo Nacional de Investigaciones Científicas y Técnicas]; Laboratorio de Invertebrados, DBBE, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II C1428EHA, Buenos Aires, Argentina
[2]CONICET; Museo Argentino de Ciencias Naturales, Av. Angel Gallardo 470, C1405DJR, Buenos Aires, Argentina
*Corresponding author: flora@bg.fcen.uba.ar

TABLE 1. *Adelomelon beckii* shell length (SL in cm) comparison between sexes in Mar del Plata populations, effects are significant at $p < 0.05$.

Variable	SL Mean 2004	SL Mean 2005	*t*-Value	df	*p*
SL_{males}	27.50	27.47	0.03	103	0.978
$SL_{females}$	31.12	30.96	0.12	125	0.904

established. For both sexes, the percentages of mature individuals were plotted against SL and SFWM. In order to estimate the size at 50% first sexual maturity, logistic regressions using nonlinear regression by least square (Statsoft, version 6.0) of SL-maturity and SFWM-maturity were used. The size and weight at which 50% of the studied population exhibit mature gonads were used as an indicator of the length at which 50% of the population became mature. Differences in SL and SFWM at maturation between sexes were tested by an analysis of covariance (ANCOVA).



FIGS. 1–4. *Adelomelon beckii*. Histological sections showing immature and mature gonads. FIG. 1: Immature gonad from a 23 cm shell length female. Digestive gland (dg), ovary (ov); FIG. 2: Mature gonad from a 29 cm shell length female, note the presence of a vitellogenic oocyte (vo); FIG. 3: Immature gonad from a 19.5 cm shell length male. Testis (T); FIG. 4: Mature gonad from a 33 cm shell length male, note the presence of spermatozoa (spz) and the seminal vesicle (sv) full of spermatozoa. Scale bars: FIG. 1 = 25 μm; FIG. 2 = 50 μm; FIG. 3 = 200 μm; FIG. 4 = 100 μm.

Considering that sexual maturation in snails produces a change in the relationship between SL and SFWM (deMaintenon, 2001), these variables were plotted. The intersection point for the pair of straight lines that best fit the data was considered the SL at which 50% of the population became mature (Somerton, 1980).

The age of first sexual maturity was calculated from the specialised von Bertalanffy growth model as described by Arrighetti (2009): SL = 405.43 * $(1 - e^{-0.098 * (t - 0.733)})$

RESULTS

No significant differences were found in SL distribution between the two reproductive seasons (2004–2005), so all data were pooled together (Table 1).

The histological analysis showed that females of *Adelomelon beckii* begin sexual maturation at 24.2 cm SL, vitellogenic oocytes in specimens less than this size were not observed (Fig. 1). SL versus maturity showed that 50%

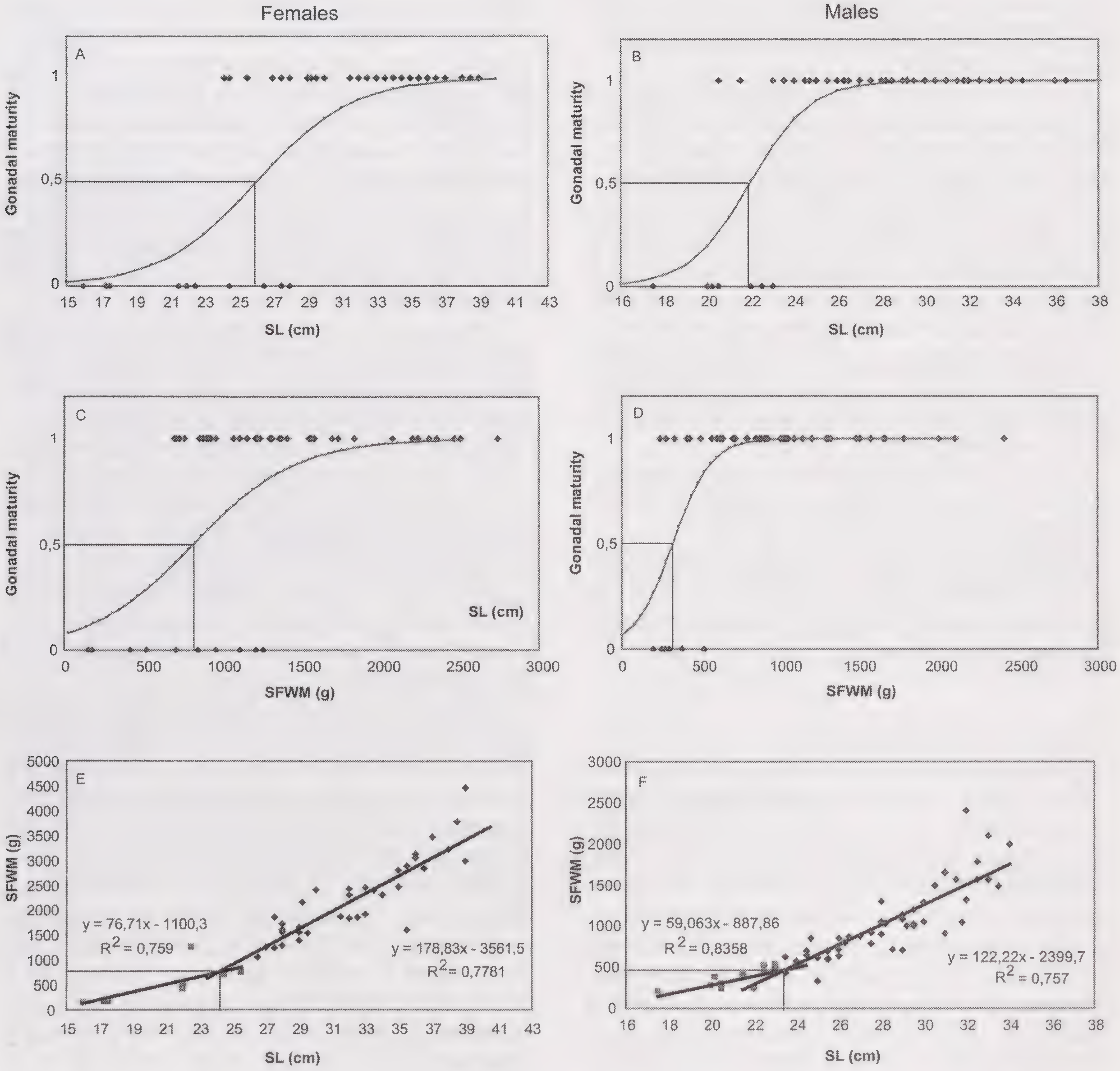


FIG. 5. *Adelomelon beckii*. Logistic regression of gonadal maturity according to histological criteria plotted against shell length (SL) for (A) females; (B) males and against shell free wet mass (SFWM) for (C) females; (D) males. 0, immature; 1, mature. SL plotted against SFWM for (E) females, (F) males, intersection between two regressions showed SL at which 50% of population became mature.

of females are mature at 25.6 cm SL (Fig. 5A). At sizes > 28 cm SL, all females were sexually mature (Fig. 2). Males did not show signs of maturation until 20.5 cm SL (Fig. 3), and below this size no spermatozoa were found in the spermatic tubules. The size at which 50% of the male population showed a mature gonad was 21.9 cm SL (Fig. 5 B). All males contained mature spermatozoa at 23 cm SL (Fig. 4). Logistic regression of SL versus maturity of gonad (according to histological criteria) follows these formulae:

$\text{Gonadal maturity}_{\text{female}}=1/(1+\exp^{(12.16-0.46*SL)})$

$\text{Gonadal maturity}_{\text{male}}=1/(1+\exp^{(15.87-0.72*SL)})$

ANCOVA of female and male size at maturation was significant $P < 0.05$.

When we analysed gonadal maturity against SFWM, the maturation process began at 690 g SFWM and 240 g SFWM for females (Fig. 5C) and males (Fig. 5D) respectively. Size at which 50% of population reaches first sexual maturity was 806.6 g SFWM for females and 321 g SFWM for males. All females were mature at > 1250 g SFWM and males at > 520 g SFWM. The following formulae were used:

$\text{Gonadal maturity}_{\text{female}}=1/(1+\exp^{(2.48-3.08*SL)})$

$\text{Gonadal maturity}_{\text{male}}=1/(1+\exp^{(2.67-8.32*SL)})$

ANCOVA of female and male SFWM at maturation was significant $P < 0.05$.

The relationship SFWM-SL showed an intersection point at 24 cm SL for females (Fig. 5D) and 23.5 cm SL for males (Fig. 5E).

Using the specialised von Bertalanffy growth model, females reach first sexual maturity at approximately 14 years, whereas males achieve this at 11 years.

DISCUSION

According to the histological criteria, females of *Adelomelon beckii*, in the sampled population, reached first sexual maturity at 25.6 cm SL, while males matured at 21.9 cm SL. Very similar results have been reported in populations of other neogastropod species, with female maturation taking place at a larger size than in males (*Buccinum cyaneum* – Miloslavich & Dufresne, 1994; *Zidona dufresnei* – Giménez & Penchaszadeh, 2003; *Adelomelon brasiliana* – Cledón et al., 2008). The later maturity of females is probably connected with their greater (compared to males) energy and materials investment during reproduction (production of egg capsules and intracapsular fluid containing proteins, carbohydrates and free amino acids (de Mahieu et al., 1974; Penchaszadeh et al., 1999). For example, in *Buccinum undatum* females invest 6 to 16 times more in reproduction than males (Brokordt et al., 2003).

When we analysed SFWM against gonadal maturity we found that 50% of the population is mature at 806.6 g SFWM for females and 321 g SFWM for males, which corresponds to 24.3 cm SL and 23 cm SL for females and males respectively. These results are similar to those found in the SL against gonadal maturity in logistic regression. The change in weight during sexual maturation is reflected in the inflexion point found in the relation of SL and SFWM, 24 cm SL for females and 23.5 cm SL for males. Our results for *A. beckii* are only applicable to the Mar del Plata population, because other studies demonstrate that size at first maturity can differ among populations from different locations (Martel et al., 1986; Kideys et al., 1993).

The age of sexual maturity is around 14 years for females and 11 years for males. This seems to be rather late if we compared other large commercial gastropods, for example, *Haliotis rubra*, six to seven years (Prince et al., 1988); *Buccinum undatum*, six to seven years (Gendron, 1992); *Zidona dufresnei*, eight years (Giménez & Penchaszadeh, 2003; Giménez et al., 2004); *Adelomelon brasiliana*, seven years (Cledón et al., 2008). Potential lifespan of *A. beckii* in Mar del Plata region is 29 years (Arrighetti, 2009), being one of the most long-lived gastropods studied to date.

Adelomelon beckii exhibits a slow growth rate (Arrighetti, 2009). This together with the lack of a planktonic opportunity for dispersal, renders this species very vulnerable to exploitation. Since the Argentinean population is currently fished, it is necessary to apply certain controls to ensure the sustainability of the stock. Considering all the analysed parameters we can conclude that 50% of female population is mature between 24 and 25.6 cm SL and 50% of male population is mature between 21.9 and 23.5 cm SL. We propose a minimum catching size of 28 cm SL for both sexes, allowing each female to spawn over at least two reproductive seasons (Arrighetti & Penchaszadeh, 2010).

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VOL. 53, NO. 1 MALACOLOGIA 2010

CONTENTS

FULL PAPERS

Julia Pizá & Néstor J. Cazzaniga
Allopatry and anatomical distinctiveness of two puzzling land snails in genus *Plagiodontes*, from Argentina (Gastropoda: Orthalicidae, Odontostominae) . 1

Andrea C. Walther, John B. Burch & Diarmaid Ó Foighil
Molecular phylogenetic revision of the freshwater limpet genus *Ferrissia* (Planorbidae: Ancylinae) in North America yields two species: *Ferrissia* (*Ferrissia*) *rivularis* and *Ferrissia* (*Kincaidilla*) *fragilis* 25

Verónica Núñez, Diego E. Gutiérrez Gregoric & Alejandra Rumi
Freshwater gastropod provinces from Argentina 47

Leandro M. Pérez, Santiago F. Genta Iturrería & Miguel Griffin
Paleoecological and paleobiogeographic significance of two new species of bivalves in the Paraná Formation (Late Miocene) of Entre Ríos Province, Argentina 61

Robert A. D. Cameron, Beata M. Pokryszko & Michal Horsák
Land snail faunas in Polish forests: Patterns of richness and composition in a post-glacial landscape 77

Wallace M. Meyer III & Robert H. Cowie
Feeding preferences of two predatory snails introduced to Hawaii and their conservation implications 135

RESEARCH NOTES

Shu H. Zhang, Qin P. Zhao, Ran Jiao, Qian Gao & Pin Nie
Identification of polymorphic microsatellites for the intermediate host *Oncomelania hupensis* of *Schistosoma japonicum* in China 147

Luke C. Strotz, Briony L. Mamo, Timothy P. Topper & Collette Bagnato
The highest southern latitude record of a living *Tridacna gigas* 155

Gabriel Mouahid, Rodrigue Mintsa Nguema, Mohamed A. Idris, Mahmoud A. Shaban, Salem Al Yafee, Juliette Langand, Marion Verdoit-Jarraya, Richard Galinier & Hélène Moné
High phenotypic frequencies of complete albinism in wild populations of *Biomphalaria pfeifferi* (Gastropoda: Pulmonata) 161

Cristián Ituarte, María Gabriela Liuzzi & Romina Centurión
Egg hull morphology in two chitons (Polyplacophora) from the southwestern Atlantic Ocean 167

María S. Avaca, Maite Narvarte & Pablo R. Martín
Description of the radula of *Buccinanops globulosus* (Neogastropoda, Nassariidae) and an assessment of its variability in northern Patagonian gulfs . 175

Katharina C. M. Heiler, Nahid Nahavandi & Christian Albrecht
A new invasion into an ancient lake – the invasion history of the dreissenid mussel *Mytilopsis leucophaeata* (Conrad, 1831) and its first record in the Caspian Sea 185

Florencia Arrighetti & Pablo E. Penchaszadeh
Size and age at first sexual maturity of the edible giant snail *Adelomelon beckii* (Neogastropoda: Volutidae) from Mar del Plata, Argentina 193

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Malacologia
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AUSTRALOCOSMICA, A NEW GENUS OF LAND SNAILS FROM THE KIMBERLEY, WESTERN AUSTRALIA (EUPULMONATA, CAMAENIDAE)

Frank Köhler

Department of Environment & Conservation, Western Australia, Science Division, Wildlife Place, Woodvale, Western Australia 6026; Australian Museum, 6 College St., Sydney, NSW 2010, Australia; frank.koehler@austmus.gov.au

ABSTRACT

The Western Australian Kimberley region harbours a diverse fauna of camaenid land snails characterised by marked patterns of narrow range endemism. A recent survey of previously poorly known areas along the Kimberley coast has resulted in the discovery of a number of further, yet undescribed camaenid taxa. One of these, the genus *Australocosmica*, is newly described herein based on comparative studies of genital anatomy, shell and radular morphology, and analyses of partial sequences of the mitochondrial marker 16S rRNA. *Australocosmica* is characterised by a broadly conical to semi-globose shell with moderately elevated spire, well-rounded, convex whorls that are separated by a deeply incised suture, and a sculpture of dense, regularly spaced axial ribs. The most distinguishing feature of the inner penial anatomy is the presence of a furrowed, collar-like vergic papillum forming the entrance of the vas deferens into the lumen of the penial chamber. Three new species, ***Australocosmia augustae***, ***A. sanctumpatriciusae***, and ***A. vulcanica***, are described based mainly on differences in penial anatomy. All species are well differentiated by uncorrected pair-wise p-distances of 15% to 20% in the 16S rRNA gene. Similar to most camaenids in the Kimberley region, species of *Australocosmica* are narrow-range endemics being restricted to single islands, sometimes including the immediately adjacent mainland coast.

Key words: Helicoidea, endemism, anatomy, mitochondrial differentiation, diversity.

INTRODUCTION

The Kimberley region of northwestern Australia is arguably one of the most remote regions of the continent. Due to its rugged landscape formed by ancient rocky ranges and the sparse infrastructure, many parts of this region, which covers an area larger than Germany, are very difficult to access. Under these conditions, it is no surprise that the documentation of the biota of vast areas has remained incomplete and that our knowledge especially with regard to the occurrence of narrow-range invertebrates is still very patchy. Camaenid land snails are such a group of animals with generally restricted ranges. In addition, these animals are difficult to collect, spending most of the year aestivating buried in the soil or hidden in rock crevices.

Morphology-based revisions have greatly contributed to a consistent taxonomy of the Western Australian Camaenidae based on comprehensive comparative analyses and have highlighted their diversity as well as marked patterns of endemism (Solem, 1979, 1981a, b, 1984). Essentially based on survey work of rainforest patches along the Kimberley coast, conducted between 1987 and 1988, Solem (1991) listed about 120 camaenid species, and a large proportion of them have remained undescribed. During an on-going biological survey conducted by the Western Australian Department of Environment and Conservation, land snails were collected on 22 larger islands off the Kimberley coast. The land snail fauna of most of these continental islands was completely unknown, and analyses of a data set of partial sequences of the mitochondrial 16S rRNA gene have demonstrated that these islands harbour substantial numbers of new candidate species that have not been recorded earlier (Köhler, 2009). These candidate species were identified by uncorrected pair-wise p-distances of at least 3% or usually much more following the approach described by Vieites et al. (2009) for the delimitation of Madagascan frog species by use of molecular markers. However, the actual taxonomic status of these candidate species awaits confirmation by morphological examination. The study of a comparatively small proportion of newly collected samples from the Kimberley Islands has indeed revealed corresponding levels of molecular and morphological differentiation in camaenids from the Kimberley both on the species and genus level. Accordingly, it has been shown that the genital anatomy provides the most reliable means to delimitate taxa. In addition, the camaenid species are generally well differentiated also on the molecular level with interspecific distances usually exceeding 10% in 16S. The on-going taxonomic work on the Camaenidae in the Kimberley has resulted in the identification of several new species of *Amplirhagada* Iredale, 1933, by Köhler (2010a) and the description of two new genera and several new species of camaenids from islands in the north Kimberley (Köhler, 2010b). However, the status of many additional undescribed taxa awaits clarification by comparative morphological studies. The present work is concerned with the study of a new genus that was preliminarily recognised by Solem (1991), who listed two potentially new genera in the Kimberley. These genera have remained unstudied and unnamed since. The novelty of one of them, referred to by Solem (1991) as “Ngen1” has recently been confirmed by a comparative study based on morphological and molecular data (Köhler, 2010b) and been described under the name *Kimberleydiscus* Köhler, 2010. It is only known from Bigge Island. The present work contains a comparative study of species of the second undescribed group referred to by Solem (1991) as “Ngen2”. Solem (1991) attributed two unnamed species to this group from the mainland of the western Kimberley near the Saint George Basin, about 400 km NE of Broome. Survey work in 2009 yielded a number of samples of additional species from the same group, as recognised by its shell, on which the present work is based.

MATERIAL AND METHODS

This study is based on ethanol preserved specimens and dry shell material collected during the Kimberley Island Survey (KIS) of the Department of Environment and Conservation, Western Australia (DEC) in 2009. Types are deposited in the Western Australian Museum, Perth (WAM) and the Australian Museum, Sydney (AM).

The observed differentiation in morphological and mitochondrial characters is herein used to formulate hypotheses on the identity of species as reproductively isolated units (i.e., to delineate species taxa) according to the biological species concept (Mayr, 1942).

Morphological descriptions focus on characters of the shell, the mantle cavity, the genital organs and the radula. Morphometric shell parameters, such as height of shell (H), diameter of shell (D), height of last whorl (LW), and width of umbilicus (U) were measured with callipers precise to 0.1 mm. The numbers of whorls of shell (N), including the protoconch, were counted as described by Barker (1999: fig. 6). All mature shells per lot were measured (as recognized by a fully developed apertural lip) as long as their spire and aperture were complete.

Anatomy of mantle and genital organs was studied using a microscope with drawing mirror. The final inking of drawings was done by Martin Püschel. Radulae and jaws were extracted manually, cleaned by soaking in 10% KOH solution for about six hours, followed by rinsing in water and ethanol. They were mounted on carbon specimen tabs for electron scanning microscopy. Radular dentition formula gives the numbers of teeth per row as follows: C (central row of teeth) + number of lateral rows of teeth + number of transitional rows of teeth + number of marginal rows of teeth. Usually, two to three specimens per sample were studied in order to ensure that the described features are consistent within taxa.

DNA was extracted from small pieces of foot muscle by use of a QIAGEN DNA extraction kit for animal tissue following the standard procedure of the manual. A fragment of the mitochondrial 16S rRNA gene (16S) was amplified was amplified by PCR using the primers 16Scs1 (Chiba, 1999) and 16Sbd1 (Sutcharit et al., 2007). Reactions were performed under standard conditions with an annealing step of 90 s at 55°C. Sequences were aligned by use of Clustal X vs. 2.0 (Larkin et al., 2007) under default settings. The aligned sequence data set comprised 5 sequences and was trimmed to a total length of 827 bp. A sequence of *Xanthomelon obliquirugosa* (E. A. Smith, 1894) was used as out-group to root the tree. The phylogenetic tree was reconstructed by application of Maximum Likelihood (ML) using the software Treefinder vs. October 2008 (Jobb et al., 2004). Prior to the ML analysis, a likelihood ratio test was performed as implemented in Treefinder to infer which model of sequence evolution fits best the data. Nodal support of the topology was inferred by calculating Expected-

TABLE 1. Shell dimensions (in mm). Given are maxima and minima (average ± standard deviation) of shell height (H), shell diameter (D), height of last whorl (LW) [each in mm], number of whorls (N), and height/diameter ratio (H/D) (n = number of measured shells).

Taxon	H	D	LW	N	H/D
A. augustae Holotype	15.3	17.5	11.5	5.2	0.87
Paratypes (n = 15)	12.7–16.3 (14.5 ± 1.1)	15.3–18.2 (16.8 ± 0.8)	9.8–12.3 (11.1 ± 0.7)	4.7–5.2 (5.0 ± 0.2)	0.80–0.93 (0.86 ± 0.04)
A. sanctumpatriciusae Holotype	13.0	15.5	9.5	4.6	0.84
Paratypes (n = 12)	11.4–13.2 (12.3 ± 0.5)	15.0–16.4 (15.9 ± 0.5)	9.2–10.4 (9.6 ± 0.3)	3.9–4.7 (4.4 ± 0.2)	0.73–0.82 (0.77 ± 0.03)
Non-types, mainland (n = 17)	9.2–12.3 (11.0 ± 0.7)	14.6–17.8 (15.6 ± 0.9)	7.8–9.5 (8.8 ± 0.4)	4.2–4.6 (4.4 ± 0.1)	0.63–0.76 (0.71 ± 0.04)
A. vulcanica Holotype	11.1	13.9	8.5	4.2	0.80
Paratypes (n = 32)	9.4–11.5 (10.4 ± 0.6)	13.1–15.0 (13.8 ± 0.4)	7.6–9.0 (8.3 ± 0.3)	3.7–4.7 (4.3 ± 0.2)	0.69–0.81 (0.75 ± 0.03)
A. spec A (n = 6)	12.9–14.6 (13.4 ± 0.6)	14.6–16.7 (15.3 ± 0.7)	9.7–11.0 (10.2 ± 0.5)	4.5–5.2 (4.7 ± 0.2)	0.81–0.95 (0.87 ± 0.04)
A. spec B (n = 2)	14.0–14.8 (14.4 ± 0.4)	17.7–17.8 (17.8 ± 0.1)	11.2–11.7 (11.5 ± 0.3)	4.6–5.6 (5.1 ± 0.5)	0.79–0.84 (0.81 ± 0.02)

Likelihood Weights of Local Rearrangements (LR-ELW) (Strimmer & Rambaut, 2002) and bootstrapping (Felsenstein, 1985), each with for 500 replicates. Pair-wise p-distances were calculated under the option 'pair-wise deletion' of gaps using the software MEGA (Tamura et al., 2007). Sequences were submitted to GenBank (Acc. Nos. HM043745-50).

Repositories: AM, Australian Museum, Sydney ; FMNH, Field Museum of Natural History, Chicago, Illinois; WAM, Western Australian Museum, Perth.

SYSTEMATIC PART

Gastropoda: Heterobranchia: Stylommatophora: Camaenidae Pilsbry, 1895

Australocosmica, gen. nov.

Type Species: ***Australocosmica augustae***, sp. nov.

Etymology: Derived from the words australe (Latin, south) and cosmica (Latin, world); of feminine gender.

Description: *Shell* between 9 to 17 mm in height and 13 to 18 mm in diameter (Table 1), with broadly conical to semi-globose shape and moderately elevated spire. Whorls wide in cross-section, convex, with well-rounded to slightly angulate periphery, slightly shouldered underneath deeply incised suture. Sculpture of pronounced, dense, regularly spaced axial ribs. Aperture simple, rounded, no traces of nodes, with thinly callued parietal wall. Outer lip thin, slightly expanded. Colour of shell yellowish brown to brownish-horn. Mantle roof with black mottled to spotted pigmentation. Vas deferens enters penial complex apically, connecting to lumen of penial chamber through a conspicuously thickened, furrowed, collar-like vergic papillum. No epiphallus present. Penis sheath well developed, comparatively thick, reaching from base to well above apex of penis. Inner penial wall may support marked longitudinal pilasters, dense pustulation, or a combination of both. Vagina more or less tubular, usually only slightly inflated. Spermatheca free, connected to spermoviduct by delicate connective tissue, reaching base of uterus. Albumen gland elongate. Talon situated within anterior quarter of albumen gland. Species aestivate as free sealers.

Comparative Remarks: Among Kimberley camaenids, somewhat similar uniformly yellowish brown, broadly conical shells with a regular, fine axial sculpture are found in

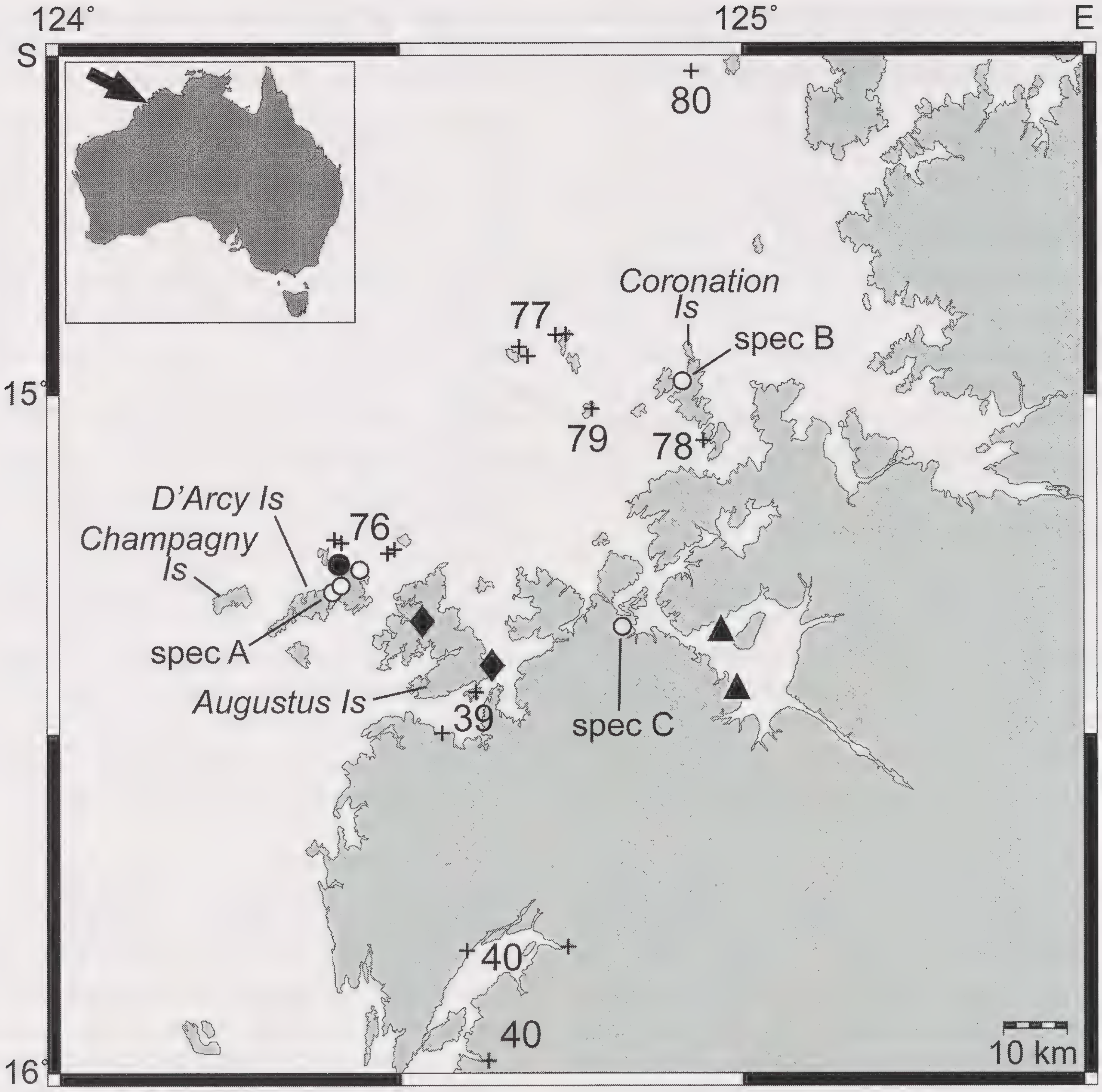

FIG. 1. Distribution map of *Australocosmica* showing occurrences of species in the northwestern Kimberley. Species described herein: ♦ – ***Australocosmia augustae***, n. sp., ▲ – ***Australocosmica sanctumpatriciusae***, n. sp., ● – ***Australocosmica vulcanica***, n. sp. Species not described herein: ○ – dry shells of *Australocosmica* spec A-C collected during the Kimberley Island Survey, + - localities of further undescribed species; material housed with the FMNH (species ID numbers and taxon delimitation by Alan Solem).

some species of *Kimboraga*, *Quistrachia*, and *Amplirhagada*. Shells of *Kimboraga* and *Quistrachia* differ by a more rounded shape, fewer and flatter whorls, not as deeply incised suture and markedly inflated body whorl. Only few *Amplirhagada* species have a similar axial sculpture, such as *A. solemiana* Köhler, 2010, and *A. indistincta* Köhler, 2010, from Middle and Southwest Osborn Islands, respectively. However, shells of *Amplirhagada* tend to be flatter (lower spire, larger diameter), which results in different H/D ratio. Their umbilicus is frequently larger and their colour lighter with brown spiral bands usually being visible. *Kimboraga*, *Quistrachia*, and *Amplirhagada* also differ in certain details of their penial anatomy. The peculiarly elevated, collar-like vergic papillum that connects the vas deferens with the lumen of the penial chamber is the most conspicuous feature differentiating species of *Australocosmica* from other Western Australian camaenids.

Distribution (Fig. 1): Currently known only from coastal portions of the mainland along the gulfs of the St. George Basin and George Water, Prince Regent Region, as well as from various islands in the York and Camden

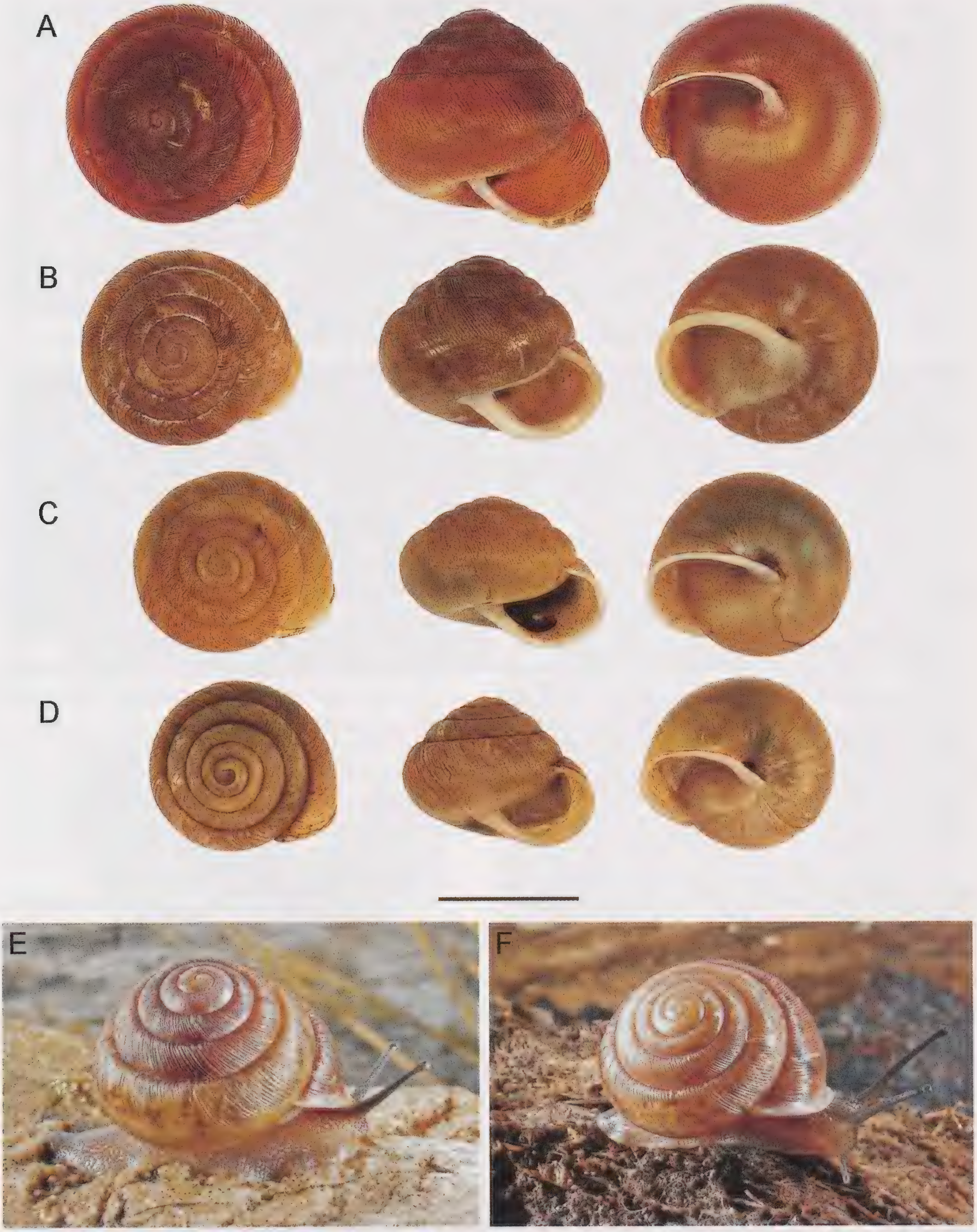

FIG. 2. Shells of the new species of *Australocosmica*, n. gen. A: Holotype of ***Australocosmica augustae***, n. sp. WAM S34667; B: Holotype of ***Australocosmica sanctumpatriciusae***, n. sp. WAM S34666; C: Shell of ***A. sanctumpatriciusae*** from the mainland opposite of St. Patrick Island WAM S49022; D: Holotype of ***Australocosmica vulcanica***, n. sp. WAM S34668. Scale bar = 10 mm; E: Living specimen of ***Australocosmica augustae***, n. sp.; F: Living specimen of ***Australocosmica sanctumpatriciusae***, n. sp. (E and F not to scale).

Sounds and Brunswick and Hanover bays off the coast. Next to the three species described here, dry shell material of species attributed to the same genus was collected at a number of other localities in this area by Vince Kessner (Adelaide River, Northern Territory), who kindly provided the locality data. Samples of additional, likely congeneric species are deposited with the Field Museum, Chicago, but were not studied by the present author.

Australocosmica augustae, sp. nov.

Type Locality: Western Australia, NW Kimberley, Bonaparte Archipelago, Camden Sound, NW section of Augustus Island, 15°20'11"S, 124°31'10"E (KIS 1-67). Vine thicket in a valley on moderately steep W facing slopes at base of sandstone cliffs; active on ground at night after rain (leg. V. Kessner, 13.02.2009).

Holotype: (Fig. 2A). WAM S34667 (preserved specimen).

Paratypes: WAM S37635 (ten preserved specimens), AM C.465175 (five preserved specimens).

Additional, non-type Material: Southern section of Augustus Island, 15°23'52"S, 124°38'30"E: WAM S 37633 (preserved juvenile), WAM S49023 (eight dried, mostly worn shells), AM C.465176 (five mostly worn shells). Augustus Island, gully in SE corner of island, 15°23'45"S, 124°37'47"E: WAM S37634 (preserved specimen).

Etymology: The species epithet refers to the name Augustus.

Description: *Shell* (Figs. 2A, 3A–D) size between about 13 and 16 mm in height and 15 to 18 mm in diameter (Table 1), broadly conical to semi-globose, with moderately elevated spire, thin (translucent) in juveniles to thick in adults. Whorls wide in cross-section, convex, with well-rounded periphery, slightly shouldered underneath deeply incised suture. Sculpture of pronounced, dense, regularly spaced axial ribs. Aperture simple, rounded, no traces of nodes, with thinly callued parietal wall, forming an angle relative to the horizontal of 45° in adults and 30° in juveniles. Outer lip thin, slightly expanded. Umbilicus completely obscured by columellar reflection or narrowly winding, about 1 mm in diameter. Colour uniformly brownish-horn, inner lip whitish. Periostracum glossy. Protoconch about 3.5 mm in diameter with 1.5 whorls, almost smooth, with very fine, indistinct axial pustulations, transition to teleoconch indistinct.

Pallial Cavity: Deep, extending about one whorl. Mantle with black mottled pigmentation. Kidney extending half length of pallial cavity, forming a narrow band.

Digestive Anatomy (Fig. 3E–I): Jaw consisting of eight regular, rather narrow plates (n = 2). Radula in average 3.2 ± 0.8 mm long with 100 ± 14 rows of teeth (31.3 ± 3.4 rows/mm) (n = 2). Tooth formula = C + 14 + 2 + 14 - 15. Central teeth with sharply pointed, slender mesocone that extends to more than half of basal plate; ectocones absent. Lateral teeth with sharply pointed, elongately ovate mesocone, about as long as base of tooth; endocones and ectocones vestigial. Marginal teeth multicuspic, mesocone and endocone similar in length, ectocone smaller than endocone, occasionally subdivided.

Genital Anatomy (Figs. 4, 5): Penis straight, of more or less of same length as anterior part of oviduct (= vagina and free oviduct). Penial retractor muscle longer than penis; attached to penial apex near entering of vas deferens. Penial sheath well developed, extending entire length, thin proximally, thick distally. No well-developed epiphallus present. Vas deferens enters penial sheath near apical portion of penial complex, forming loop or coil before opening to penial lumen via thick-

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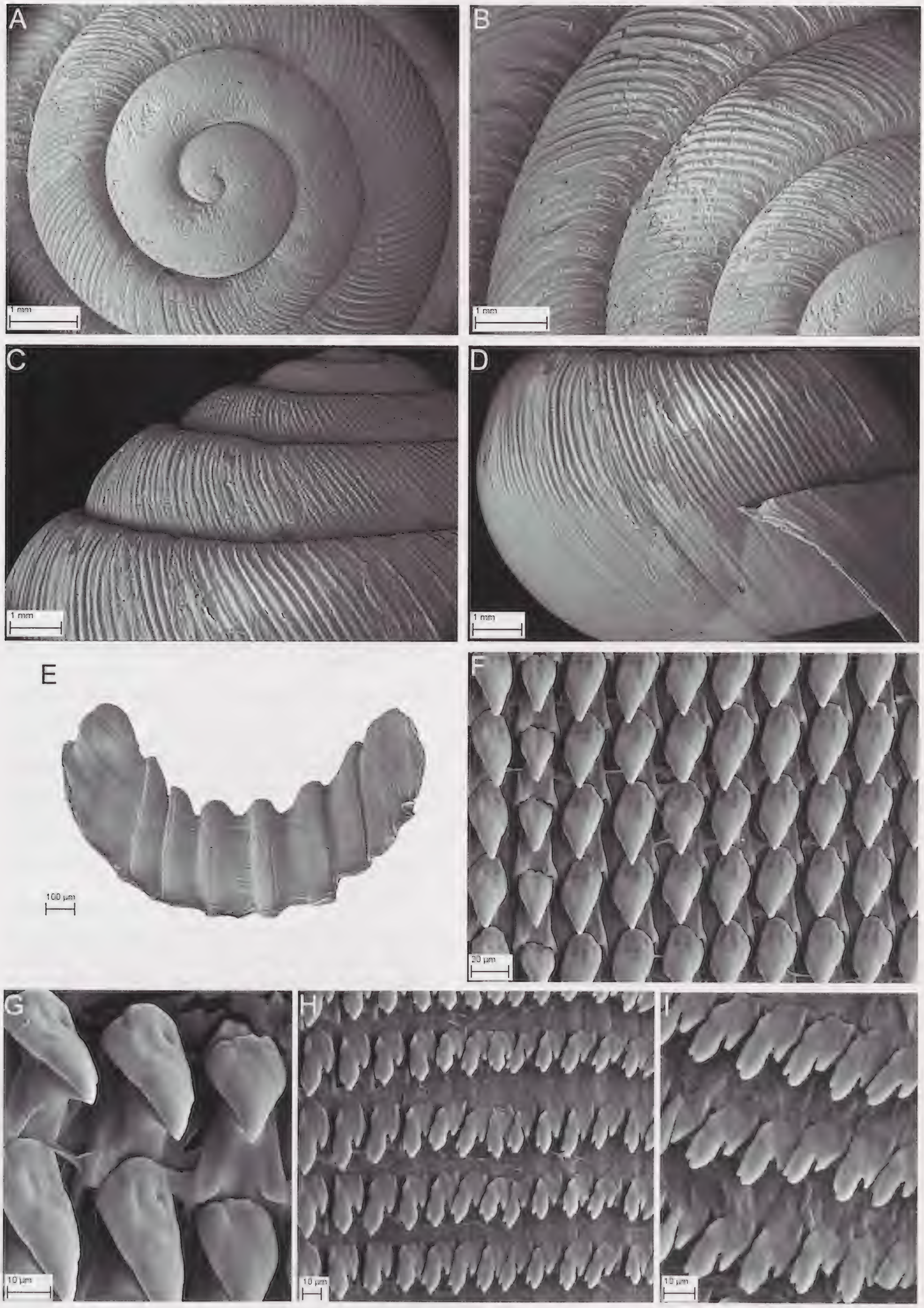
FIG. 3. SEM photographs of ***Australocosmica augustae***, n. sp. A–D: Shell, paratype AM C.465175, scale bars = 1 mm; A: Apical view; B: Details of 2nd to 4th whorl viewed from above; C: Front view; D: Front view showing last whorl and part of aperture; E: Jaw, paratype AM C.465175. Scale bar = 100 µm; F–I: Radula, paratype AM C.465175; F: Central and inner lateral teeth viewed from above. Scale bar = 20 µm; G: Close-up of central and inner lateral teeth; H: Inner and central marginal teeth viewed from above; I: Close-up of central marginal teeth. Scale bars = 10 µm.


A
1 mm
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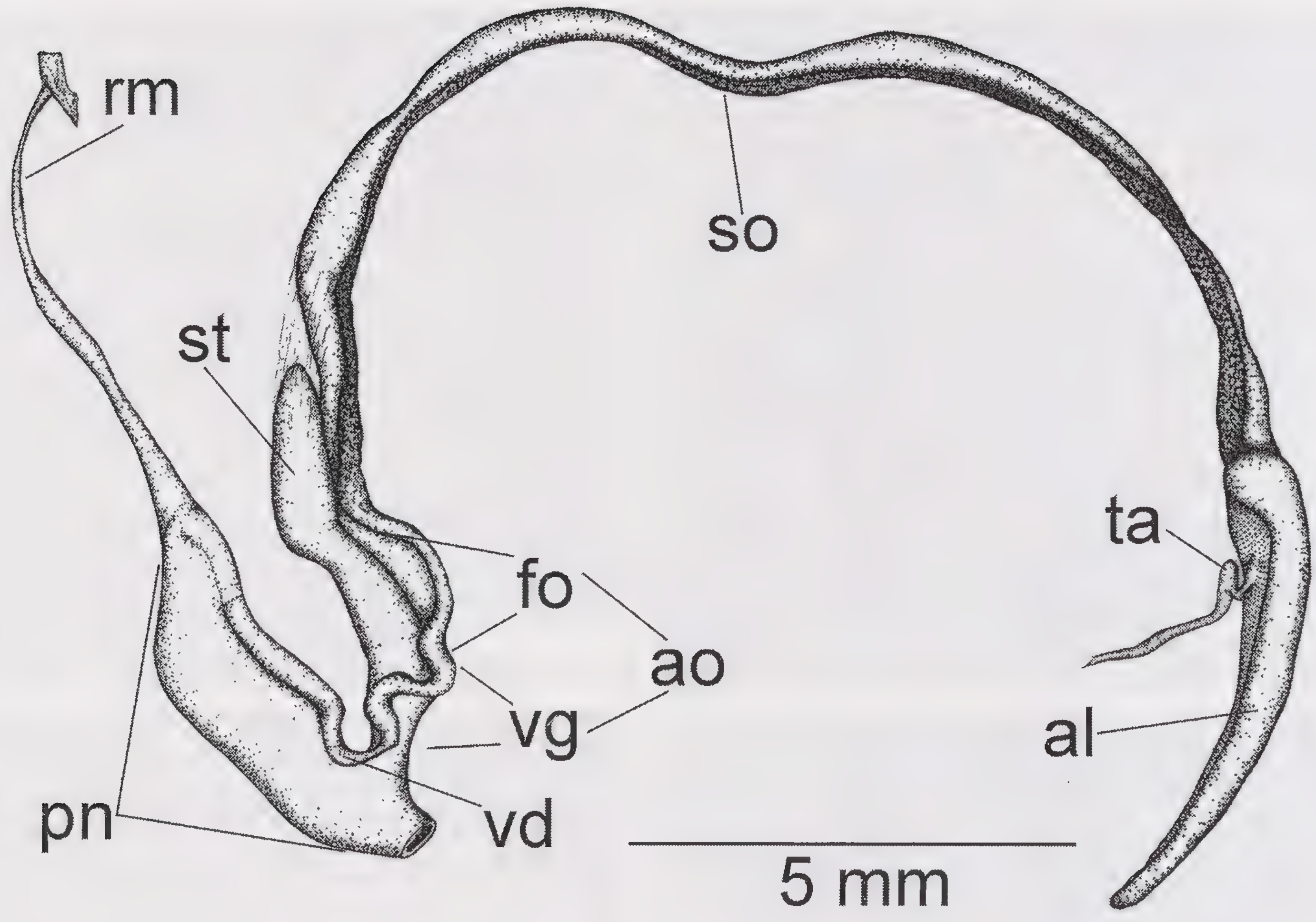

FIG. 4. Genital anatomy of ***Australocosmica augustae***, n. sp., paratype AM C.465175. Scale bar = 5 mm. Abbreviations: al – albumen gland, ao – anterior part of oviduct, fo – free oviduct, pn – penis, rm – retractor muscle, so – spermoviduct, st – spermatheca, ta – talon, vd – vas deferens, vg – vagina.

ened, furrowed, collar-like vergic papillum. Inner penial wall smooth. Two longitudinal pilasters form along entire length of the penis wall on each side of opening to vas deferens; additional, weaker pilaster underneath this opening shorter.

Vagina moderately long, posteriorly inflated. Inner wall of vagina and anterior part of oviduct with two or three smooth longitudinal pilasters. Spermatheca short, connected with spermoviduct by delicate connective tissue, reaching distal base of spermoviduct; duct and head slightly differentiated; duct wide, with two smooth longitudinal pilasters; head elongately inflated with longitudinal pilasters, delicate wall. Free oviduct (the part of the oviductal between vagina and the fused spermoviduct), short, more or less straight, about same length as vagina. Spermoviduct much longer than anterior part of oviduct. Albumen gland elongate. Talon situated within first quarter of albumen gland, not embedded in albumen gland; hermaphroditic duct tightly undulating.

Comparative Remarks: ***Australocosmica augustae*** is the type species of the genus. For comparisons with congeneric species, see descriptions below.

Australocosmica sanctumpatriciusae, sp. nov.

Type Locality: Western Australia, NW Kimberley, Saint George Basin, St. Patrick Island, 15°21’23”S, 124°57’55”E (KIS 2-64). Vine thicket on steep slopes, sandstone boulders, under rocks in loose soil (leg. V. Kessner, 09.02.2009)

Holotype (Fig. 1B): WAM S37666 (preserved specimen).

Paratypes: WAM S37637 (two preserved specimens, shells cracked). WAM S49021 (six dried, mostly worn shells), AM C.465173 (five dried, mostly worn shells).

Additional, non-type Material: Kimberley mainland, small peninsula ca. 8 km SE of St. Patrick Island, 15°26’02”S, 125°00’23”E: WAM S37638 (three preserved specimens), WAM S49022 (13 dried, mostly worn shells), AM C.465174 (five dried, mostly worn shells).

Etymology: The species epithet refers to the name of Saint Patrick (Sanctus Patricius, in Latin), the name patron of St. Patrick Island.

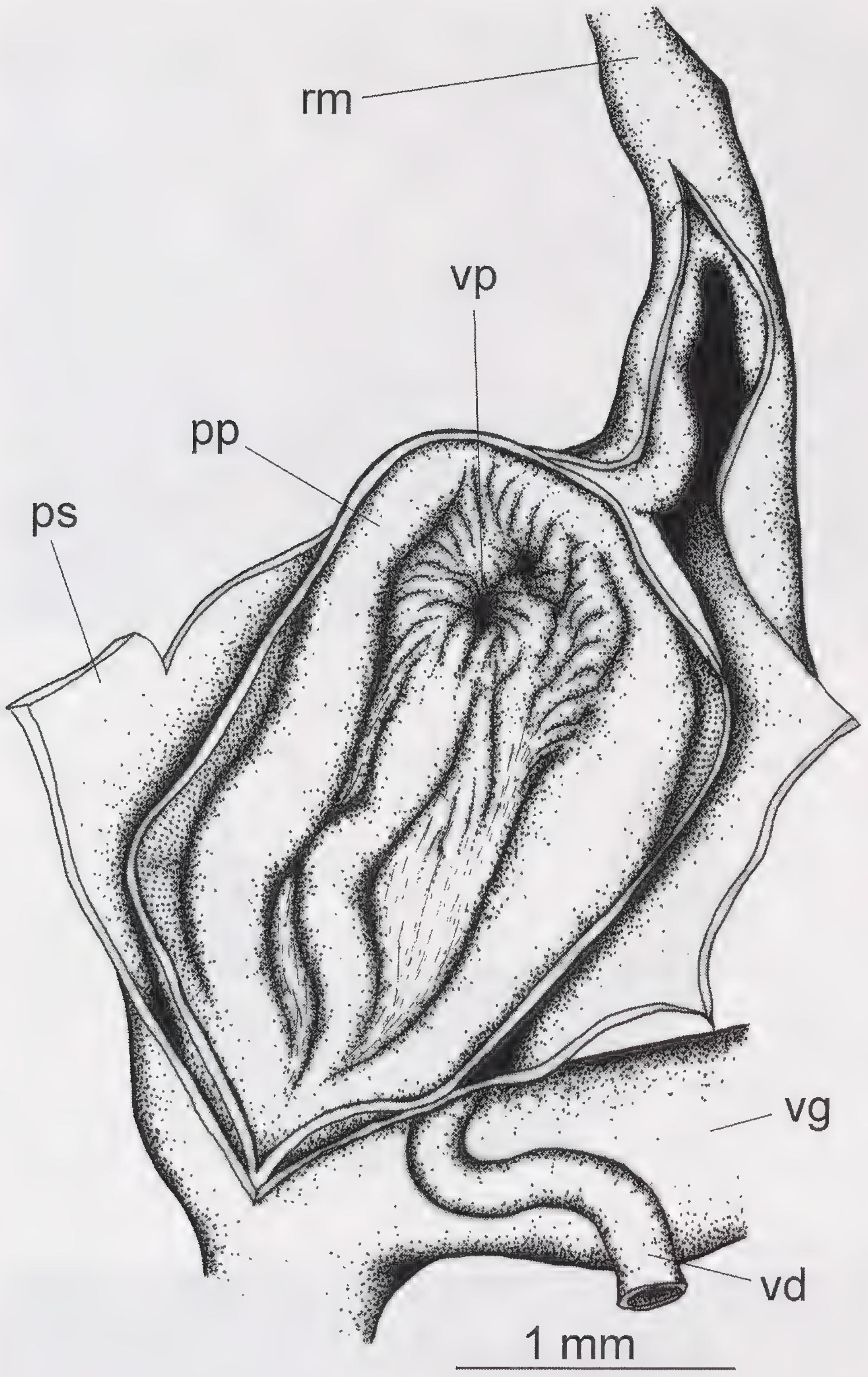

FIG. 5. Penial anatomy of ***Australocosmica augustae***, n. sp., paratype AM C.465175. Scale bar = 1 mm. Abbreviations: pp – penial pilaster, ps – penial sheath, rm – retractor muscle, vd – vas deferens, vg – vagina, vp – vergic papillum.

Description: *Shell* (Figs. 1B, 6A–C) size between about 11 and 13 mm in height and 15 to 16 mm in diameter (Table 1), broadly conical to semi-globose, with moderately elevated spire, translucent in juveniles to thin in adults. Whorls moderately wide in cross-section, convex, with well-rounded to slightly angulate periphery, slightly shouldered underneath deeply incised suture. Sculpture of very thin, dense, regularly spaced axial ribs. Aperture simple, rounded, no traces of nodes, with thinly callued parietal wall, forming an angle relative to horizontal of 60°. Outer lip thin, very slightly expanded. Umbilicus almost entirely covered by columellar reflection, forming a chink. Colour uniformly dark hornish-brown, inner lip whitish. Periostracum glossy. Protoconch about 2.5 mm in diameter, with about 1.5 whorls, almost smooth, with very fine, indistinct axial pustulations, transition to teleoconch indistinct.

Pallial Cavity: Extending about half a whorl. Mantle pigmented with black spots. Kidney extending about half of pallial cavity.

Digestive Anatomy (Fig. 6D–F): Jaw consisting of eight to nine regular, narrow plates (n = 1). Radula 3.8 mm long with 115 rows of teeth (30.6 rows/mm) (n = 1). Tooth formula = C + 14 + 3 + 16. Central teeth with sharply pointed, slender mesocone that extends to more than half of basal plate; ectocones vestigial. Lateral teeth with pointed, elongately ovate mesocone, about as long as base of tooth; endocones and ectocones vestigial. Marginal teeth multicuspic, mesocone and endocone similar in length, ectocone smaller than endocone, occasionally subdivided.

Genital Anatomy (Figs. 7, 8): Penis straight, more or less as long as anterior part of oviduct. Penis retractor muscle about as long as penis; attached to penial apex near entering of vas deferens. Penial sheath well developed, extending entire length, thin proximally, thick distally. No well-developed epiphallus present. Vas deferens entering penial sheath near apical portion of penial complex, forming loop or coil before opening to penial lumen via conspicuous furrowed, collar-like vergic papillum. Proximal end of inner penial wall with obliquely arranged, fine, irregular transversal folds being distally replaced by large, rhomboid pustules that comprise most of inner penis wall. A well-developed, corrugated pilaster forming underneath opening to vas deferens.

Vagina moderately long, posteriorly inflated. Inner wall of vagina and anterior part of oviduct with many delicate longitudinal pilasters. Spermatheca short, connected with spermoviduct by delicate connective tissue, reaching base of spermoviduct; spermatheca duct and head only slightly differentiated; duct wide, with many delicate longitudinal pilasters; head elongate, with longitudinal pilasters; wall of head delicate. Free oviduct short, slightly shorter than vagina. Spermoviduct much longer than anterior part of free oviduct. Albumen gland short. Talon within first quarter of albumen gland, not embedded in albumen gland. Hermaphroditic duct tightly undulating.

Comparative Remarks: Specimens from the mainland (listed under Additional, non-type Material) with slightly smaller shells and narrowly winding umbilicus exhibit a similar pe-

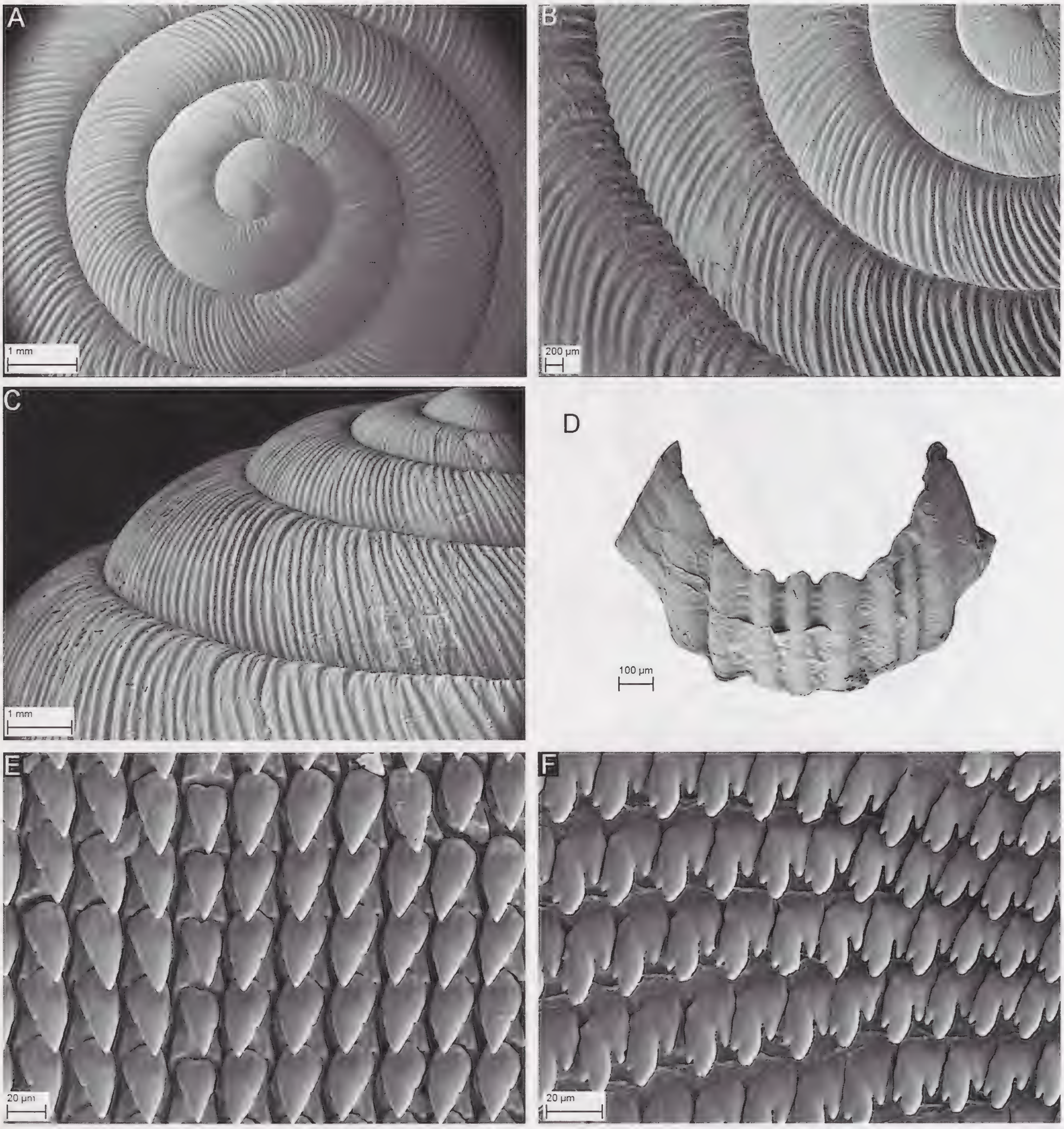

FIG. 6. SEM photographs of ***Australocosmica sanctumpatriciusae***, n. sp. A–C: Shell, paratype AM C.465174; A: Apical view, scale bar = 1 mm; B: Details of 2nd to 4th whorl viewed from above, scale bar = 200 µm; C: Front view, scale bar = 1 mm; D: Jaw, paratype WAM S37637, scale bar = 100 µm; E, F: Radula, paratype WAM S37637, scale bars = 20 µm; E: Central and inner lateral teeth viewed from above; F: Close-up of inner to central marginal teeth.

nial morphology and are genetically not well differentiated. In absence of clearly separating features, they are treated as conspecific with the population on St. Patrick Island. The shell of this species is somewhat intermediate between those of ***A. augustae*** and ***A. vulcanica*** with respect to shape and size (Table 1). It tends to be smaller and flatter than ***A. augustae***, and larger than ***A. vulcanica*** (at least shells from the type locality). Umbilicus of types forms a chink as in ***A. augustae*** but unlike ***A. vulcanica***. However, the shells from the mainland have a narrowly open umbilicus. The most distinctive feature of this species is the dense pustulation of the entire penial wall and the presence of a corrugated pilaster underneath the opening of the vas deferens into the lumen of the penial chamber.

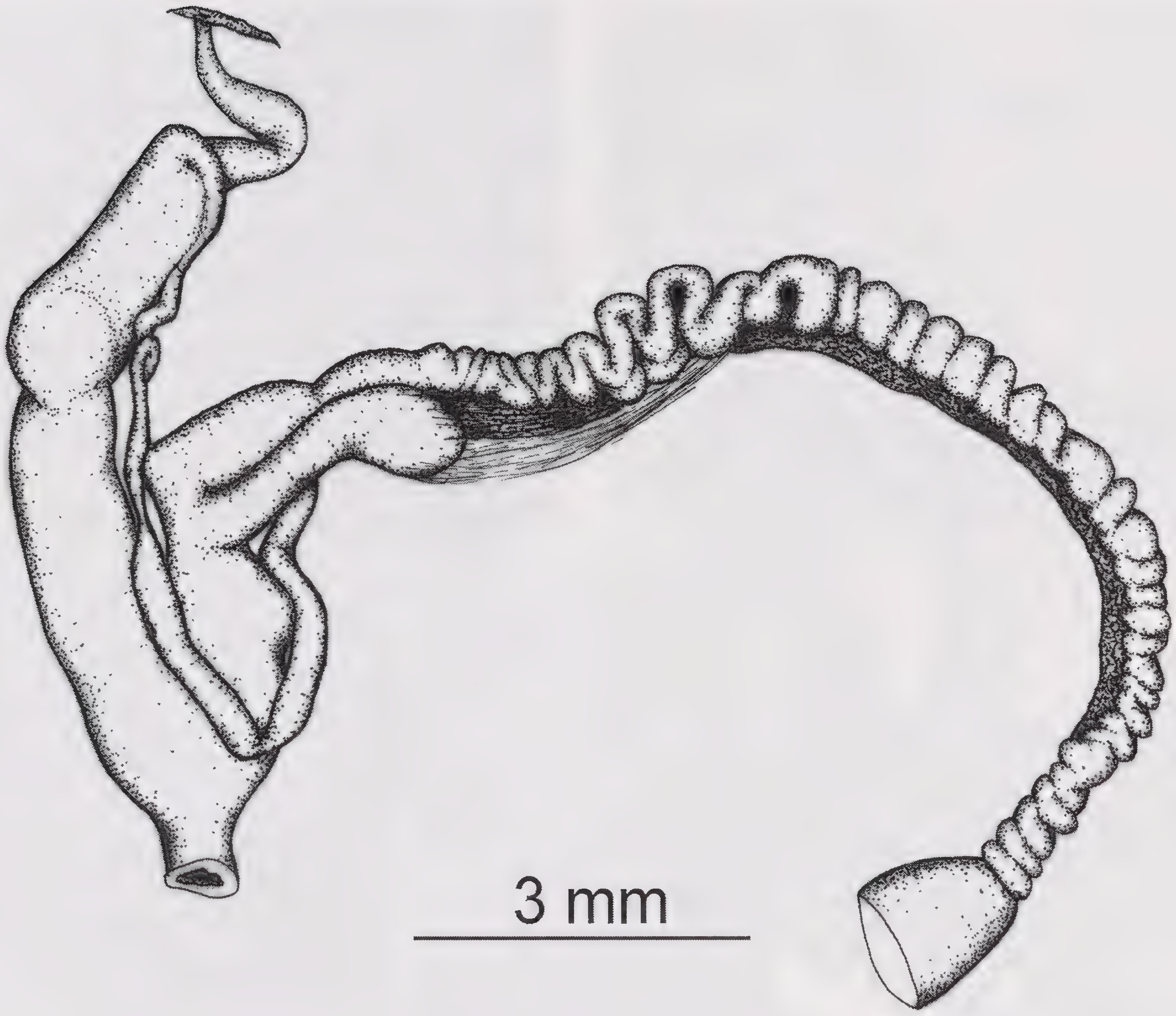

FIG. 7. Genital anatomy of ***Australocosmica sanctumpatriciusae***, n. sp., paratype WAM S37637. Scale bar = 5 mm. For labelling of the organs see Fig. 4.

Australocosmica vulcanica, sp. nov.

Type Locality: Western Australia, NW Kimberley, Bonaparte Archipelago, Unnamed Island in Vulcan Islands Group, 1.4 km from NE corner of D'Arcy Island, 15°15'26"S, 124°24'22"E (KIS 2-63). Vine thicket on volcanic scree above rocky beach, in leaf litter amongst boulders; common (leg. V. Kessner, 04.02.2009)

Holotype (Fig. 1C): WAM S37668 (preserved specimen).

Paratypes: WAM S37636 (18 preserved specimens, 3 shells cracked), AM C.465177 (five preserved specimens), WAM S49020 (13 dried, mostly worn shells), AM C.465178 (ten dried, mostly worn shells).

Etymology: The species epithet refers to the Vulcan Island group, where this species is found to dwell on volcanic rocks. The Latin word '*vulcano*' is derived from 'Vulcan', the name of the Roman god of fire.

Description: *Shell* (Figs. 1C, 9A–D) size between about 9 and 11 mm in height and 13 to 15 mm in diameter (Table 1), broadly conical to semi-globose, with moderately elevated spire, translucent in juveniles to thin in adults. Whorls moderately wide in cross-section, convex, with well-rounded to slightly angulate periphery, slightly shouldered underneath deeply incised suture. Sculpture of very thin, dense, regularly spaced axial ribs. Aperture simple, rounded, no traces of nodes, with thinly callued parietal wall, forming an angle relative to horizontal of 60°. Outer lip thin, very slightly expanded. Umbilicus narrowly open to forming a chink. Colour uniformly yellowish brown, inner lip whitish. Periostracum glossy. Protoconch about 2.5 mm in diameter with about 1.5 whorls, almost smooth, with very fine, indistinct axial pustulations, transition to teleoconch indistinct.

Pallial Cavity: Extending about half a whorl. Mantle pigmented with black spots. Kidney extending less than half of pallial cavity.

Digestive Anatomy (Fig. 9E–H): Jaw consisting of eight regular, narrow plates (n =

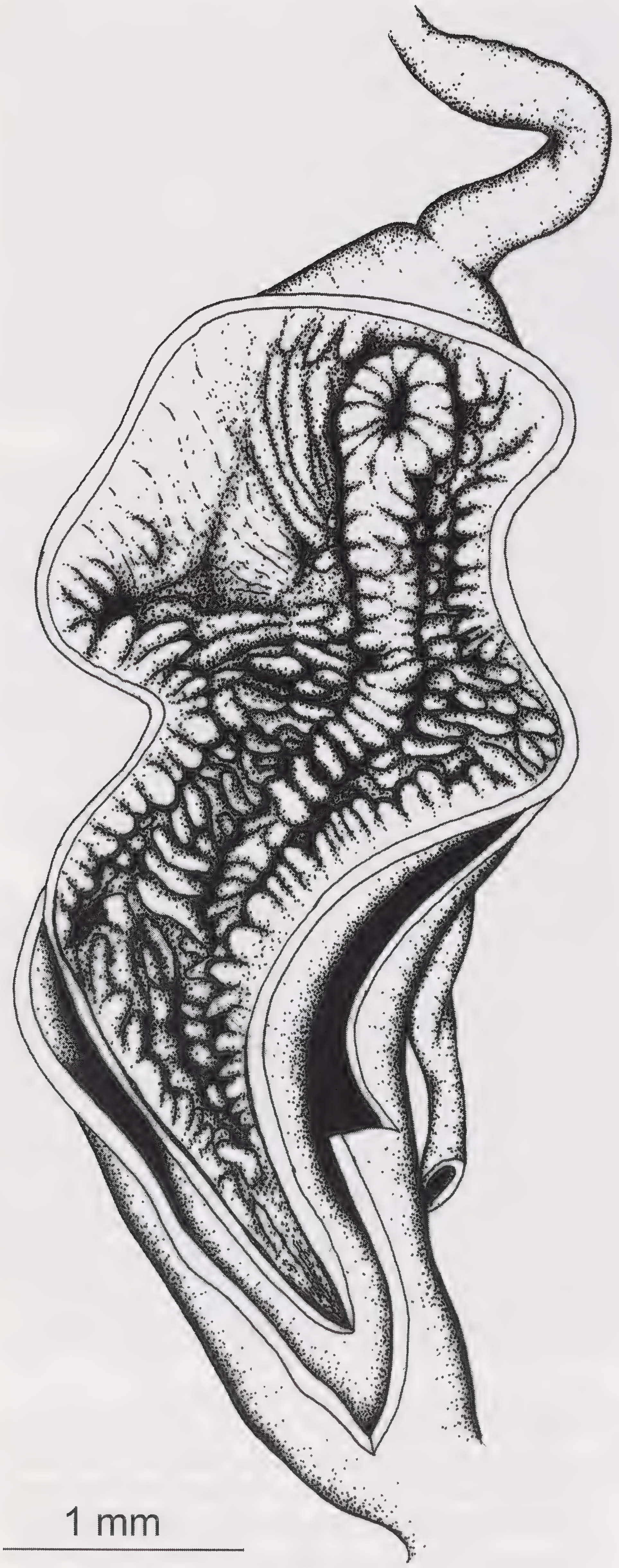

FIG. 8. Penial anatomy of ***Australocosmica sanctumpatriciusae*** n. sp., paratype WAM S37637. Scale bar = 1 mm. For labelling of the organs see Fig. 5.

2). Radula 4.3 ± 0.4 mm long with 128 ± 21 rows of teeth (30 ± 2 rows/mm) (n = 2). Tooth formula = C + 14 + 2 - 3 + 14 - 16. Central teeth with sharply pointed, slender mesocone that extends to more than half of basal plate; ectocones absent. Lateral teeth with pointed, elongately ovate mesocone, about as long as base of tooth; endocones and ectocones vestigial. Marginal teeth multicuspic, mesocone and endocone similar in length, ectocone smaller than endocone, occasionally subdivided.

Genital Anatomy (Figs. 10, 11): Penis strongly inflated; clearly shorter than anterior part of oviduct. Penis retractor muscle about as long as penis; attached to penial apex near entering of vas deferens. Penial sheath well-developed, extending entire length, thin proximally, thick distally. No well-developed epiphallus present. Vas deferens enters penial sheath near apical portion of penial complex, forming loop or coil before opening to penial lumen via thickened, furrowed, collar-like vergic papillum. Parts of inner penial wall pad-liked thickened, smooth, delimited by two thick, smooth longitudinal pilasters; distal part with dense pustulation; pustules large, irregularly arranged.

Vagina moderately long, tubular. Inner wall of vagina and anterior part of oviduct with two to three smooth longitudinal pilasters. Spermatheca short, reaching base of spermoviduct, entirely connected with spermoviduct by delicate connective tissue; spermatheca duct and head only slightly differentiated; duct wide, with two smooth longitudinal pilasters; head elongately inflated, with longitudinal pilasters, delicate wall. Free oviduct shorter than vagina. Spermoviduct longer than anterior part of oviduct. Albumen gland elongate. Talon within first quarter of albumen gland, not embedded. Hermaphroditic duct tightly undulating.

Comparative Remarks: Shell smaller, flatter, with finer sculpture than ***A. augustae*** and smaller than ***A. sanctumpatriciusae*** (Table 1). Umbilicus narrowly winding instead of being entirely covered by columellar reflection as in ***A. augustae***. Differs from its congeners by inflated penis proper and pad-like thickening of inner penial wall.

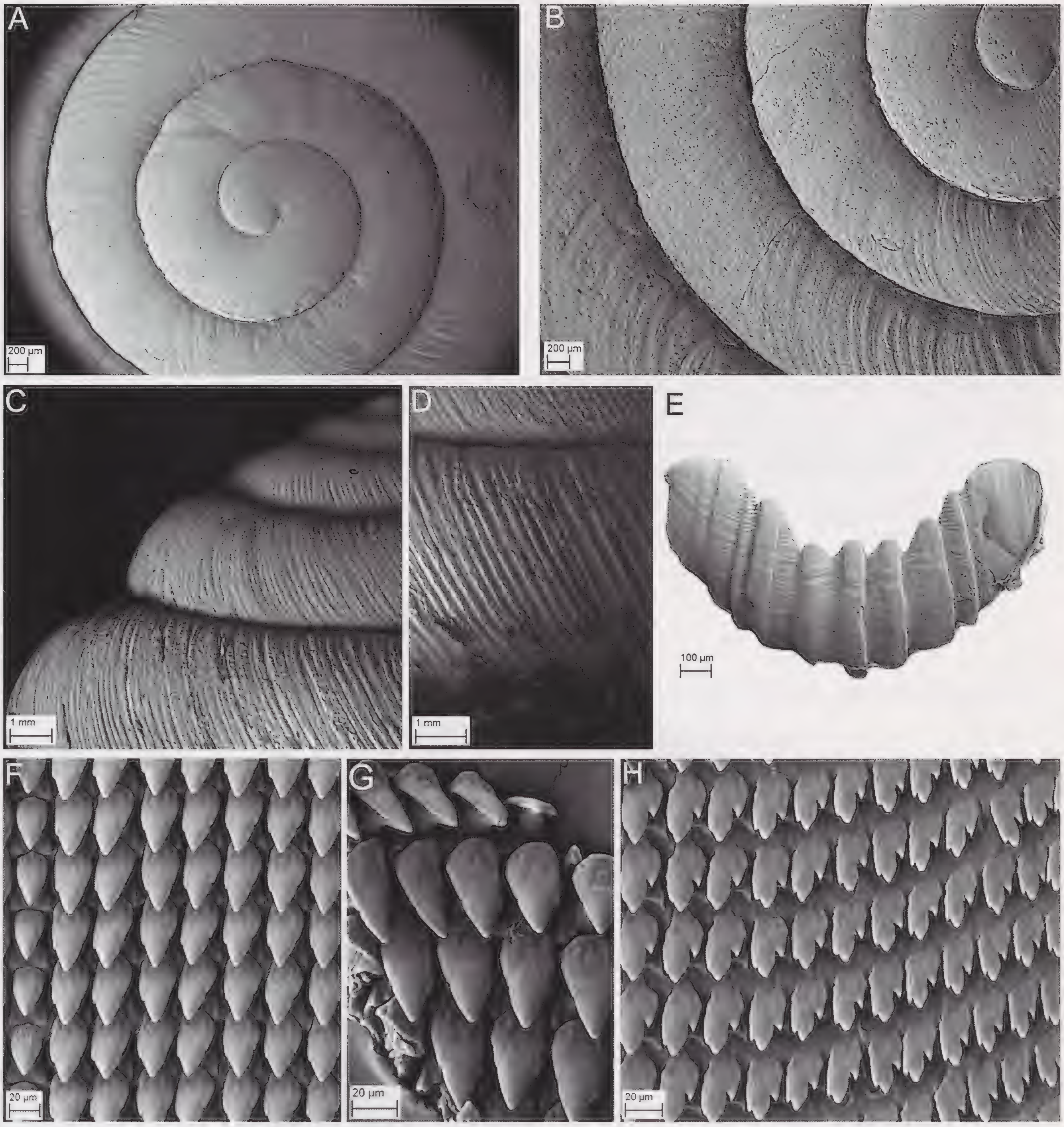

FIG. 9. SEM photographs of ***Australocosmica vulcanica***, n. sp. A–D: Shell, paratype AM C.465178; A: Apical view, scale bar = 200 µm; B: Details of 2nd to 4th whorl viewed from above, scale bar = 200 µm; C: Front view, scale bar = 1 mm; D: Close-up of last whorl; E: Jaw, paratype AM C.465177, scale bar = 100 µm; F–H: Radula, paratype AM C.465177, scale bars = 20 µm; F: Central and inner lateral teeth viewed from above; G: Close-up of central and inner to lateral teeth; H: Inner and central marginal teeth.

Australocosmica spec. A

Dry shells: WAM S37162 (Western Australia, NW Kimberley, Bonaparte Archipelago, D'Arcy Island, 15°15'30"S, 124°26'25"E), WAM S37160 (D'Arcy Island, 15°17'29"S, 124°24'00"E).

Comparative Remarks: Shells from D'Arcy Island differ from their geographically closest congener, ***A. vulcanica***, by larger size and more elevated spire (Table 1). Since no preserved material is available, their taxonomic status remains questionable.

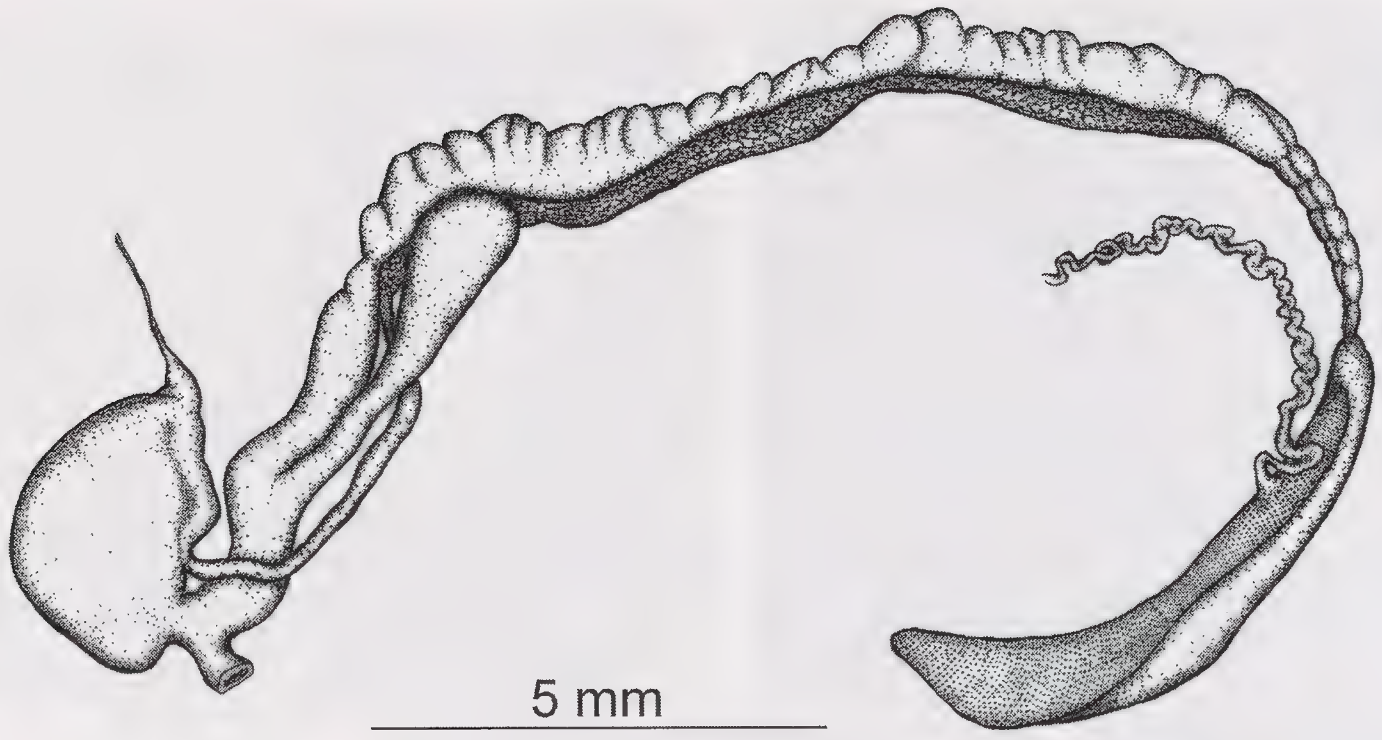

FIG. 10. Genital anatomy of ***Australocosmica vulcanica***, n. sp., paratype AM C.465177. Scale bar = 5 mm. For labelling of the organs, see Fig. 4.

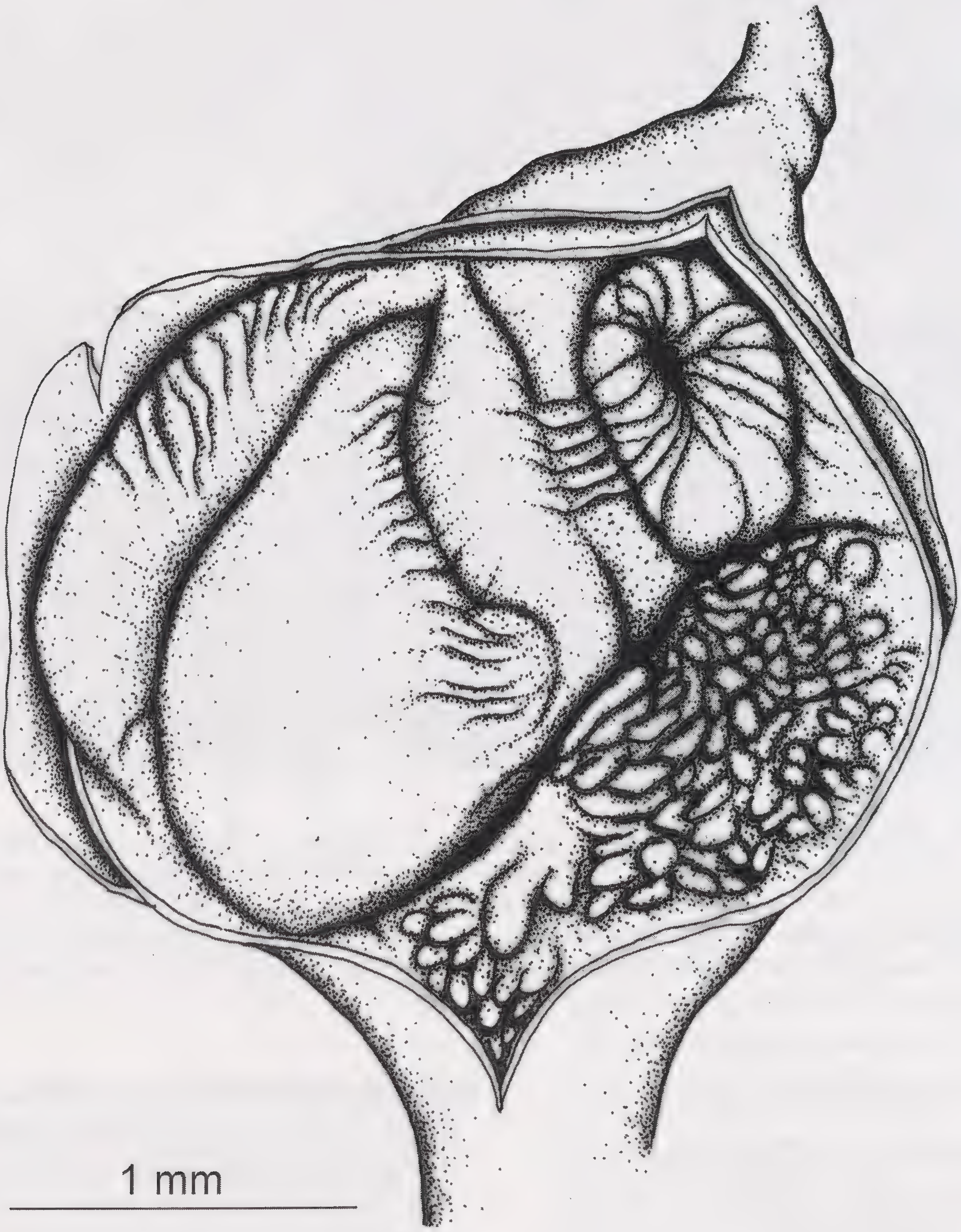

FIG. 11. Penial anatomy of ***Australocosmica vulcanica***, n. sp., paratype AM C.465177. Scale bar = 1 mm. For labelling of the organs, see Fig. 5.

Australocosmica spec. B

Dry, worn shells: WAM S37159 (Western Australia, NW Kimberley, Bonaparte Archipelago, Northern section of Coronation Island, 14°58'48"S, 124°54'46"E). WAM S37158 (Northern section of Coronation Island, 14°58'35"S, 124°54'47"E).

Comparative Remarks: Five worn and leached shells, larger than all other species, closest to ***A. augustae*** regarding size and shape. Given their isolated occurrence most likely representing an undescribed species.

Australocosmica spec. C

Dry, worn shells: WAM S49035 (Western Australia, NW Kimberley, mainland, 1.3 km S of halfway Bay, Munster Water, 15°20'15"S, 124°49'23"E).

Comparative Remarks: Three worn and leached shells; average in size and shape, of uncertain affinities.

Molecular Analyses

An about 800 bp-long fragment of the mitochondrial gene 16S was sequenced from one or two specimens per species in order to infer the rate of genetic divergence. Analyses of a comprehensive data set of about 500 sequences representing more than 175 species of all known camaenid genera from Western Australia have confirmed the monophyly of the new genus *Australocosmica* as delineated herein. This phylogeny, as first shown by Köhler (2009), will be published elsewhere. Here I analysed only five sequences of *Australocosmica* in order to infer the levels of genetic differentiation among species. The likelihood ratio test revealed the general time reversible model of sequence evolution (GTR+i+Γ) as the best-fit model. The Maximum Likelihood phylogram revealed all species to represent well-differentiated clades (Fig. 12). Pair-wise comparisons of sequences showed that species were differentiated by means of 15% to 20% uncorrected p-distances, whereas conspecific specimens differed by less than 1%. The present phylogenetic tree has a very restricted taxon sampling and is largely inconclusive with regard to the relationships among the three recognized species. ***Australocosmica sanctumpatriciusae*** is shown at an unresolved, basal position, wheras the two other species are shown in a sister group relationship. However, this relationship receives little nodal support in terms of LR-ELW values.

DISCUSSION

Levels of Morphological and Molecular Differentiation

In his revisions, Solem (1979, 1981a, b, 1984) developed a classification of the Western Australian Camaenidae based on features of the shell and genital anatomy. He found that especially penial anatomy is informative on both the species and genus level. So far, the morphology-based classification has not been tested by use of molecular data. However, it has recently been confirmed for *Amplirhagada*, a species-rich genus of Western Australian camaenids, that species usually possess a distinctive penial anatomy, while shell features not always provide reliable means to differentiate species (Köhler, 2010a). *Amplirhagada*

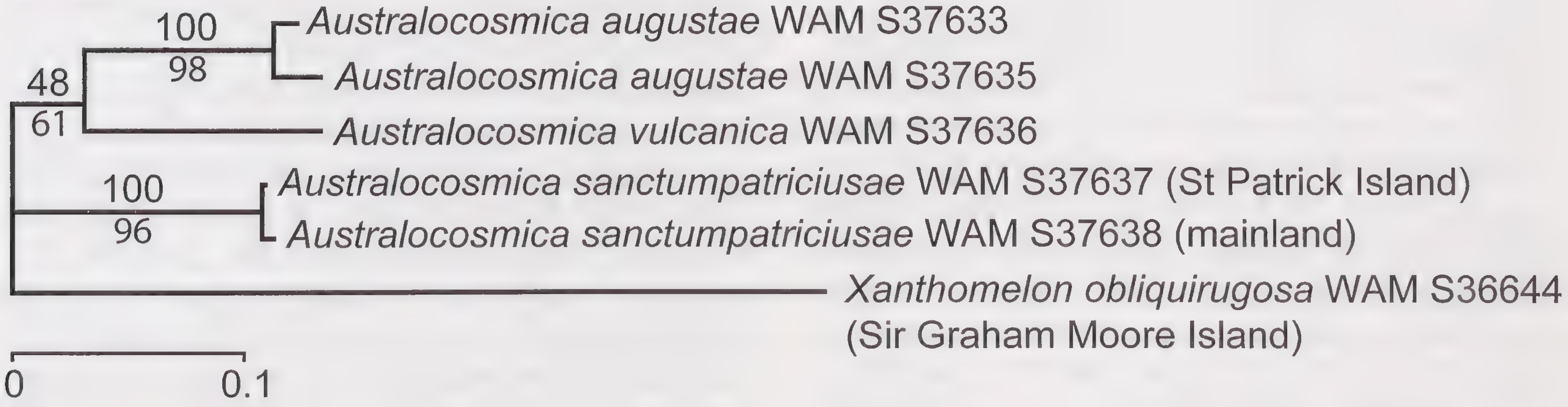
100
98
48
61
Australocosmica augustae WAM S37633
Australocosmica augustae WAM S37635
Australocosmica vulcanica WAM S37636
100
96
Australocosmica sanctumpatriciusae WAM S37637 (St Patrick Island)
Australocosmica sanctumpatriciusae WAM S37638 (mainland)
Xanthomelon obliquirugosa WAM S36644 (Sir Graham Moore Island)
0
0.1

FIG. 12. Maximum likelihood phylogram based on analysis of partial sequences of the mitochondrial gene 16S rRNA. Numbers on branches indicate nodal support by Expected-Likelihood Weights of Local Rearrangements (LR-ELW); numbers below branches indicate bootstrap support.

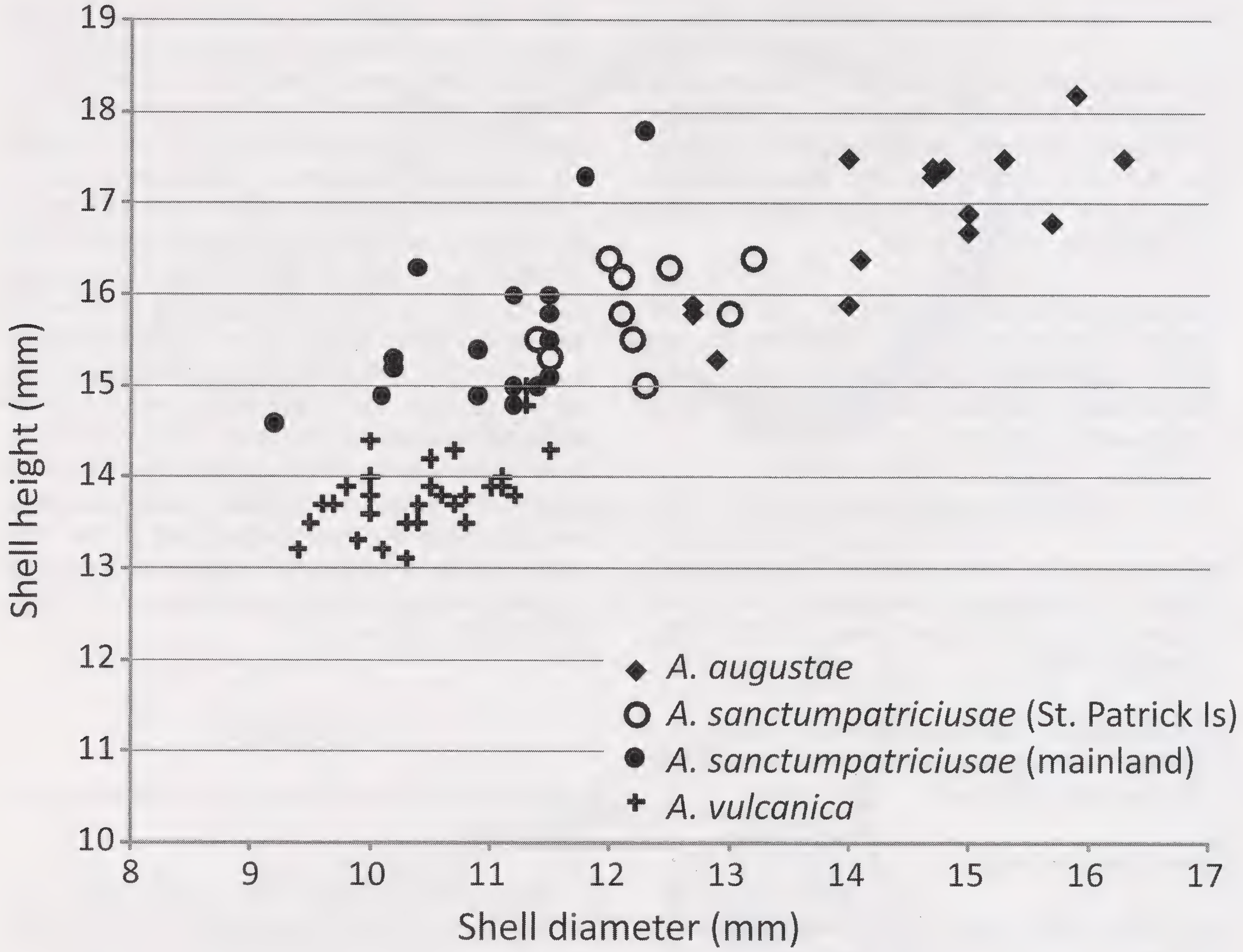

FIG. 13. Morphometrical chart comparing shells of the three newly described species of *Australocosmica* by means of their height and diameter (in mm).

species circumscribed with emphasis on penial anatomy were also found to be characterised by considerable levels of genetic differentiation in mitochondrial markers (of up to 30% Tamura-Nei distances in the cytochrome c oxidae gene, COI). A phylogeny of the Western Australian Camaenidae revealed comparable levels of sequence divergence also for 16S (Köhler, 2009). In addition, corresponding levels of morphological and mitochondrial differentiation were also observed in camaenid species of *Torresitrachia* Iredale, 1939, from limestone outcrops in the Northern Territory (Willan et al., 2009). These two systematic studies mainly addressed the levels of differentiation between species by using only few representative specimens of each species. However, a study that included a large number of specimens from various populations of *Amplirhagada* from 16 islands in the Kimberley showed that the genetic variation within populations was generally low, while populations from different islands were always separated by considerable genetic distances (Johnson et al., 2010). This finding underscores the utility of mitochondrial markers for species recognition in this group of land snails. In contrast to mitochondrial data and penial anatomy, shell morphology has not been as informative on the species level because of a large overlap of the variation within and between populations and species. Therefore, there is evidence from several groups that in Western Australian Camaenidae levels of morphological (with respect to penial anatomy) and molecular differentiation are closely linked, while shells are less informative. The observations in *Australocosmica* do well correspond with these findings. Species recognised by their penial anatomy are also well differentiated in the mitochondrial marker 16S. By contrast, while species differ to some degree in size and shape of their shells, they also show considerable variation within populations and a large overlap in the morphological ranges of species (Fig. 13). This is most conspicuously demonstrated for ***A. sanctumpatriciusae***, which has a comparatively small shell with narrowly open umbilicus on St. Patrick Island and larger shells with an umbilicus entirely covered by the columellar reflection on the mainland. Consequently, shell morphology is mainly informative at the generic level whereas it is of low taxonomic value at the infrageneric level.

Diversity and Distribution of *Australocosmica*

The monophyly of *Australocosmica* has received high nodal support in a comprehensive phylogeny of the Western Australian Camaenidae (Köhler, 2009). It was therefore not of concern here. *Xanthomelon* was used as out-group, because it was found to be member of a clade of taxa that forms the sister group of *Australocosmica*. The considerable genetic distances between all taxa included in the sequence data set may have caused long-branch attraction. The lacking support for the monophyly of *Australocosmica* in the tree presented here is attributed to this phenomenon. It is not considered to indicate problems with the correct delineation of the genus *Australocosmica*.

The distribution of *Australocosmica* is apparently restricted to the coastal regions and offshore islands of the northwestern Kimberley region in or near the Prince Regent Reserve. Mapping of all available distribution data, which includes also undescribed species found here and listed by Solem (1991), suggests that the genus occurs roughly between the York Sound in the north and the Collier Bay in the south (Fig. 1). In addition to the three species described here, there are several further species that were preliminarily delineated by Alan Solem under manuscript names but were not studied in detail. Because Western Australian Camaenidae usually are narrow range endemics (Solem, 1991), the manuscript species listed by him likely are further distinct species. These are likely endemic on Albert (spec. 80), Coronation (spec. 78), Buffon (spec. 77), Maret (spec. 79), and D'Arcy islands (spec. 76) (including satellite islets), as well as on the mainland opposite of Augustus Island (spec. 39) and around the Walcott Inlet (spec. 40). As by now, the relation of three described to possibly seven to ten undescribed species of *Australocosmica* underscores that there is a considerable proportion of formally unknown species diversity in the Kimberley with the number of undescribed species actually exceeding that of the described species. This holds not only true for *Australocosmica* but probably also for other genera in the group.

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EVALUATION OF A NEW MOLLUSCICIDE FOR COUNTERACTING THE INTERMEDIATE SNAIL HOST OF *SCHISTOSOMA JAPONICUM*

Yi Yuan[1, 2], Huifen Dong[1*], Xingjian Xu[2*], Guiling Li[3], Fenghua Wei[2], Yunbing Zhao[3], Zuwu Tu[2], Min Liu[3], Mumin Cao[2], Hui He[2], Li Tang[2], Hong Zhu[2] & Hongping Fan[2]

ABSTRACT

A novel molluscicide, derived from niclosamide, the salt of quinoid-2',5-dichloro-4'-nitrosalicylanilide (LDS) was recently developed in China. The molluscicidal activity of LDS was compared with the commonly used molluscicide niclosamide (WPN) by immersion at seven concentrations; 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L, and with spraying and powdering methods (dosages of 0.2, 0.4, and 0.8 g/m^2) in the laboratory and the field. We recorded mortality at 1 d, 3 d, and 7 d. The results showed that at 0.4 mg/L immersion exposure for 72 hours, snail mortalities for LDS and WPN were 100% and 96.70% respectively in the lab, and 100% and 95.33 ± 1.15% respectively in the field. With a dosage of 0.8 g/m^2 exposure for 7 d by spraying, snail mortalities for LDS and WPN were 100% and 97.33 ± 2.30% respectively in the lab, and 99.27 ± 1.27% and 97.47 ± 3.11% in the field. With a dosage of 0.8 g/m^2 exposure at 7 d by powdering, snail mortalities for LDS and WPN were both 98% in the lab, and 100% in the field. These results from three different methods show that molluscicidal effects were similar in the lab and the field. However, LDS is much cheaper than WPN, and LDS is less toxic to fish than WPN. Therefore, LDS might be more useful than WPN for controlling snails in endemic areas of schistosomiasis in China.

Key words: China, *Oncomelania*, schistosomiasis, molluscicide, niclosamide, WPN, LDS, snail control.

INTRODUCTION

Schistosoma japonicum is a serious epidemic disease in East and Southeast Asia, including China, Indonesia, Japan and Philippines. *Oncomelania hupensis* is the unique snail intermediate host of *Schistosoma japonicum*. Currently, the major commercial chemical molluscicide niclosamide (WPN) is applied in schistosomiasis foci for snail control worldwide (MOH, 2000; Takougang et al., 2007; Lv et al., 2006; Coura-Filho et al., 1992; Meyer-Lassen et al., 1994; Graebing et al., 2004a). Since WPN was recommended by the World Health Organization (WHO, 1985, 1993, 2002), a 50% wettable powder WPN is commonly used to kill *Oncomelania hupensis* snails in China (Mao, 1990; Zhou et al., 2005; NSMC, 2006). However, due to WPN's shortcomings of considerable cost to control snails and also toxicity to fish, and the fact that it precipitates rapidly, application of WPN has been restricted in some economically poor areas and aquaculture sites in China. Recently, there has been considerable exploratory research on molluscicides involving both chemicals and plants (Zhu, 1984; Liu et al., 2000; Wei et al., 2001; Xu et al., 2003, 2004; Yuan et al., 2005a). A new molluscicide named LDS, the salt of quinoid-2',5-dichloro-4'-nitrosalicylanilide, derived from niclosamide, has been developed. The purpose of this paper is to compare the molluscicidal effects of LDS and WPN.

MATERIAL AND METHODS

Experimental Materials

Molluscicides: A 10% wettable powder of LDS was obtained from the Tongji Medical School, Huazhong University of Science and Technology. The 50% wettable powder of niclosamide ethanolamine salt (WPN) was purchased from

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[1] Department of Medical Parasitology, School of Basic Sciences, Wuhan University, Wuhan, Hubei 430071, China
[2] Hubei Institute of Schistosomiasis Control, Zhou Daoquan Bei Road No. 6, Wuhan, Hubei 430079, China
[3] Huazhong University of Science and Technique, Tongji Medical College, China
*Corresponding authors: hfdong@whu.edu.cn; xuxj0612@yahoo.com.cn

TABLE 1. Percent mortality for time and concentration of two drugs investigated by immersion tests in the lab.

Active Concentration (mg/L)	LDS			WPN		
	24 h	48 h	72 h	24 h	48 h	72 h
0.05	5.57 ± 5.10	25.57 ± 1.96	33.33 ± 3.35	10.00 ± 3.30	35.93 ± 2.37	48.90 ± 15.42
0.1	15.57 ± 1.96	58.90 ± 6.97	94.43 ± 1.96	15.57 ± 1.96	44.43 ± 1.96	87.77 ± 5.08
0.2	27.80 ± 1.91	87.77 ± 12.64	98.90 ± 1.91	22.20 ± 1.91	63.33 ± 3.35	92.23 ± 3.87
0.4	64.77 ± 2.54	100.00 ± 0.00	100.00 ± 0.00	35.97 ± 2.39	70.20 ± 0.35	96.70 ± 0.00
0.8	86.37 ± 0.29	100.00 ± 0.00	100.00 ± 0.00	42.20 ± 1.91	95.57 ± 1.96	100.00 ± 0.00
1.6	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	46.70 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3.2	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	56.70 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control	2.87 ± 0.51	3.53 ± 0.68	5.70 ± 1.00	2.87 ± 0.51	3.53 ± 0.68	5.70 ± 0.00

the Sichuan Academy of Chemical Industry and Design.

Test Containers: Unglazed earthen bowls (20 cm diameter and 7 cm height) were used. Soil containing rich humus and other organic materials was collected from the snail habitats. It was air-dried then ground into powder. We mixed 500 g powdered soil with 500 ml water to make a thick soil paste about 2 cm thickness, which was placed all over the bottom of each earthen bowl. The snail can produce eggs in the soil paste.

Porcelain enamel trays (35 cm x 45 cm x 1,575 cm) were used to test the efficacy of spraying and powdering. Powdered soil (2,000 g) was used to make a 3–5 cm thick soil paste covering the bottom of each tray. The soil was kept in a 30% wet state by dewatering with 90% ethanol and subsequently bringing to a 30% wet state by adding water. Aquaria (15 cm diameter and 12 cm height containing 2 L test solution was used in the lab to test for toxicity to fishes.

Animals: Adult *O. hupensis* (5.5 to 7.5 mm in length) were collected locally and used as experimental animals. After being rinsed in dechlorinated tap water and kept for 24 h to acclimate in the lab, snails with higher vitality and similar size (presumably of similar age) were chosen for the experiments.

Snail Eggs: 300 experimental snails were kept in earthenware bowls (25 cm diameter and 10 cm height) for 24 h in order to lay eggs. After 24 h, all adult snails were moved from the bowls. These bowls were then kept at room temperature for 25 d. At 25 d, all newly hatched young were removed and unhatched eggs were collected using a 40-mesh sieve (0.45/380 μm).

Zebra fish of 2–3 cm length were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. These fish were acclimated for one week before testing in aquaria.

Experimental Methods

The molluscicidal effects of LDS were assessed by immersion, spraying, and powdering methods in the lab and field. We also used WPN as the molluscicide control and blank control with dechlorinated tap water in the lab and natural water in the field. All lab experiments were done at a room temperature of 25 ± 1°C.

TABLE 2. Results of analysis of variance for two drugs investigated by immersion tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	39,464.21	19,732.11	1,589.60	< 0.0001
Molluscicide	1	3,438.85	3,438.85	264.57	< 0.0001
Concentration	7	133,292.52	19,041.79	1,465.01	< 0.0001
Error of measurement	64	794.45	12.41	-	-

FIGS. 1–6. Application of molluscicide against *Oncomelania hupensis*. FIG. 1: Immersion in the laboratory; FIG. 2: Immersion in the field; FIG. 3: Spraying in the laboratory; FIG. 4: Spraying in the field; FIG. 5: Powdering in the laboratory; FIG. 6: Powdering in the field.

TABLE 3. Changing trend over time for two drugs investigated by immersion tests in the lab.

Resources of Variance	Time	v	SS	MS	F	P
Time	Linearity	1	37,036.37	37,036.33	429.58	< 0.001
Time* Molluscicide	Linearity	1	2,698.76	2,698.76	31.30	< 0.001
Time* Concentration	Linearity	7	12,646.98	1,806.71	20.96	< 0.001
Error of measurement	Linearity	39	3,362.43	86.22	-	-

TABLE 4. LC_{50} of two drugs at different times.

	LDS			WPN		
Exposure Time (h)	LC_{50}	95% Confidence Interval		LC_{50}	95% Confidence Interval	
24	0.4751	0.2989	0.7758	0.2470	0.1914	0.3148
48	0.0691	0.0517	0.0854	0.0903	0.0692	0.1122
72	0.0568	0.0472	0.0657	0.0400	0.0188	0.0547

Snail Immersion: In the lab, 30 active, mature *O. hupensis* were exposed to LDS and WPN at seven concentrations 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L (Fig. 1). Snail mortalities were observed after 24 h, 48 h, and 72 h respectively. In the control group, the same number of snails was kept in dechlorinated tap water under the same conditions. Mortality was determined by crushing the snails (MOH, 2000). LC_{50} values were calculated by probits.

In the field, seven irrigation and drainage ditches (30 m x 2 m) were selected. Nine nylon bags, each with 30 active mature *O. hupensis*, were used. Three nylon bags were immersed in each concentration group of 0.2, 0.4, 0.8 mg/L. This was repeated for LDS and WPN (Fig. 2). Snail mortality in each trial was observed after 24 h, 48 h, and 72 h.

Snail Spraying: In the lab, the concentrations of LDS and WPN were the same as in the immersion method. Molluscicides were sprayed on each tray (1,000 mL/m^2 dechlorinated tap water with dissolved LDS and WPN respectively) (Fig. 3). Two hundred active mature *O. hupensis* were thus exposed for 1 d, 3 d, and 7 d in the trays. After each such exposure, 50 snails were collected randomly to assess mortality.

In the field, 2,100 m^2 of snail habitat on the Yangtze River flood plain was selected and divided into seven sections. Weeds and vegetation were cut down before spraying. In each section, three groups of *O. hupensis* were sprayed with LDS and WPN, each at concentrations of 0.2 g/m^2, 0.4 g/m^2, and 0.8 g/m^2 (Fig. 4). After 1 d, 3 d, and 7 d, 200 snails were collected randomly in each section to identify mortality.

Snail Powdering: In the lab, concentrations of LDS and WPN were as for the immersion method. The chemicals were mixed with sand (1:4) and the mixture was scattered over each tray (Fig. 5). 200 active mature *O. hupensis* were exposed for 1 d, 3 d, and 7 d in earthenware trays. After 1 d, 3 d, and 7 d, 50 snails were collected to determine mortality.

In the field, a similar terrain and vegetation was selected as for the spraying method. LDS and WPN were mixed with fine sand (at the same concentration as above) before scattering the powder (Fig. 6). For each section, the area used was 300 m^2. After 1 d, 3 d, and 7 d, 200 snails were collected randomly in each section to identify mortality.

Snail Egg Immersion: The concentration of LDS and WPN was the same as in with the immersion method. Fifty snail eggs were put in each 50 mL beaker. The beakers were filled with 50 mL of molluscicide solution for each concentration group. The snail eggs were

TABLE 5. Percent mortality for time and concentration of two drugs investigated by immersion tests in the field.

	LDS			WPN		
Active Concentration (mg/L)	24 h	48 h	72 h	24 h	48 h	72 h
0.2	44.00 ± 2.00	94.67 ± 1.15	100.00 ± 0.00	28.00 ± 2.00	68.67 ± 1.15	90.00 ± 0.00
0.4	91.33 ± 3.06	99.33 ± 1.15	100.00 ± 0.00	62.00 ± 2.00	76.00 ± 2.00	95.33 ± 1.15
0.8	95.33 ± 1.15	100.00 ± 0.00	100.00 ± 0.00	75.33 ± 3.06	95.33 ± 1.15	100.00 ± 0.00
Control	4.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	4.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

TABLE 6. Results of analysis of variance for two drugs investigated by immersion tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	7,522.11	3,761.06	1,962.29	< 0.0001
Molluscicide	1	2,244.50	2,244.50	1,496.33	< 0.0001
Concentration	7	89,152.17	29,717.39	19,811.60	< 0.0001
Error of measurement	32	61.33	1.92	-	-

removed from the solutions after exposure for 24 h, 48 h, and 72 h and then cleaned with dechlorinated tap water. After a 48 h hatching period, the usual hatching time for *O. hupensis*, hatching rate and viability were determined.

Fish Toxicity: The half-static state method (NSMC, 1995) was used to assess toxicity. The concentration of LDS and WPN was the same as in the immersion method. Ten fish were exposed in each concentration for 8 h, 24 h, 48 h, 72 h, and 96 h and the mortality at each time was recorded. Fish death was recorded when a fish floated on the surface of the water and did not move even after stimulating the fish tail with a needle. LC_{50} was calculated by probits.

Cost Comparison: The cost comparison of LDS with WPN was done. The unit cost and the total dosage needed to treat the same snail habitats of one hectare were compared for the two molluscicides.

Statistical Methods

Statistical analysis was performed with SAS statistical software, version 8.02. The *P* value accepted for statistical significance was $P < 0.05$. Analysis of variance (ANOVA) was used to analyze repeated measurement data.

RESULTS

Snail Immersion: In the lab, there was significantly greater mortality using LDS at 24 h and 48 h when the concentration of the chemicals was 0.4 mg/L (24 h, LDS vs. WPN, t = 14.313, $P < 0.001$; 48 h, LDS vs. WPN, t = 149, $P < 0.001$), but there was no significant difference at 72 h (Table 1).

At 72 h, all the snails were dead and at concentrations above 0.4 mg/L. There was 100% mortality at each time interval. All three factors (time, molluscicide and concentration) showed significant differences (Table 2).

Snail mortality increased with time and concentration, exposure time getting longer and concentration increasing (Table 3). LC_{50} vales for immersion at room temperature (25 ± 1°C), LC_{50} for 48 h exposure, LDS was 0.0691 mg/L. WPN was 0.0903 mg/L (Table 4).

TABLE 7. Percent mortality for time and concentration of two drugs investigated by spraying tests in the lab.

	LDS			WPN		
Active Concentration (g/m^2)	1 d	3 d	7 d	1 d	3 d	7 d
0.05	32.67 ± 15.28	48.67 ± 11.55	71.33 ± 12.86	31.33 ± 15.28	44.67 ± 11.55	65.33 ± 16.28
0.1	45.33 ± 11.02	75.33 ± 12.70	87.33 ± 5.77	45.33 ± 12.86	66.67 ± 16.77	78.00 ± 10.00
0.2	51.33 ± 16.17	88.00 ± 7.21	92.00 ± 5.29	51.33 ± 16.29	75.33 ± 11.02	84.00 ± 10.00
0.4	65.33 ± 11.02	95.33 ± 4.16	97.33 ± 2.31	63.33 ± 16.29	83.33 ± 9.24	89.33 ± 5.77
0.8	79.33 ± 7.57	100.00 ± 0.00	100.00 ± 0.00	79.33 ± 11.02	95.33 ± 2.31	97.33 ± 2.30
1.6	86.67 ± 8.08	100.00 ± 0.00	100.00 ± 0.00	94.00 ± 3.46	100.00 ± 0.00	100.00 ± 0.00
3.2	95.33 ± 2.31	100.00 ± 0.00	100.00 ± 0.00	97.33 ± 4.62	100.00 ± 0.00	100.00 ± 0.00
Control	2.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00

TABLE 8. Results of analysis of variance for two drugs investigated by spraying tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	7,522.11	3,761.06	1,962.29	< 0.0001
Molluscicide	1	2,244.50	2,244.50	1,496.33	< 0.0001
Concentration	3	89,152.17	29,717.39	19,811.60	< 0.0001
Error of measurement	32	61.33	1.92	-	-

In the field, The two-sample t test showed that the difference between LDS and WPN were statistically significant at 24 h and 48 h, (24 h, LDS vs. WPN, t = 14.914, *P* < 0.001; 48 h, LDS vs. WPN, t = 17.500, *P* < 0.001) and displayed no statistical significance in 72 h (72 h, LDS vs. WPN, t = 7.00, *P* = 0.02) (Table 5). All results are statistically significant for factors of time, molluscicide and concentration (Table 6).

Snail Spraying: In the lab, the result of two-sample t tests showed there were no statistical differences between LDS and WPN at 1 d, 3 d, and 7 d (1 d, LDS vs. WPN, t = 0, P = 1.000; 3 d, LDS vs. WPN, t = 3.500, P = 0.073 and 7 d, LDS vs. WPN, t = 2.00, P = 0.184) (Table 7).

Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 8).

In the field, two-sample t test showed that the differences between LDS and WPN were statistically significant at 24 h and 48 h (24 h, LDS vs. WPN, t = 4.541, *P* = 0.01; 48 h, LDS vs. WPN, t = 2.789, *P* = 0.049) but not at 72 h (Table 9). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 10).

Snail Powdering: In the lab, the results of two-sample t tests showed that the differences between LDS and WPN were not statistically significant at 1 d, 3 d, and 7 d (1d, LDS vs. WPN, t = 0, *P* = 1.000; 3 d, LDS vs. WPN, t = 1.265, *P* = 0.275 and 7 d, LDS vs. WPN, t = 0, *P* = 1.000) (Table 11). Time and concentration showed no statistically singificant variation, whereas mollusicide did show such a variation (Table 12).

In the field, two-sample t tests showed statistically significant differences between LDS and WPN at 1 d (LDS vs. WPN, t = 4.541, *P* = 0.01) and 3 d (LDS VS WPN, t = 2.789, *P* = 0.049) but not at 7 d (LDS vs. WPN, t = 0.929, *P* = 0.406) (Table 13). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 14). As seen in Table 14, that all of them had statistical significance at the level of time, molluscicide, and concentration.

Snail Egg Immersion: When *O. hupensis* eggs were exposed to LDS and WPN at concentrations of 0.8 mg/L for 24 h, 48 h, and 72 h, two-sample *t* tests showed that the differences between LDS and WPN were statistically significant at 24 h (24 h, LDS vs. WPN,

TABLE 9. Percent mortality for time and concentration of two drugs investigated by spraying tests in the field.

Active Concentration (g/m^2)	LDS			WPN		
	1 d	3 d	7 d	1 d	3 d	7 d
0.2	21.03 ± 1.63	78.77 ± 6.47	90.53 ± 1.53	19.17 ± 1.69	60.20 ± 5.04	92.07 ± 1.53
0.4	55.63 ± 2.95	91.77 ± 5.02	96.53 ± 3.23	52.80 ± 6.08	82.40± 5.00	95.77 ± 1.06
0.8	91.80 ± 4.23	96.63 ± 2.97	99.27 ± 1.27	72.20 ± 6.16	91.30 ± 1.47	97.47 ± 3.11
Control	3.57 ± 0.31	4.43 ± 0.40	4.33 ± 0.23	3.57 ± 0.31	4.43± 0.40	4.33 ± 0.23

TABLE 10. Results of analysis of variance for two drugs investigated by spraying tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	13,624.06	6,812.03	799.09	< 0.0001
Molluscicide	1	429.25	429.25	27.00	< 0.0001
Concentration	3	80,511.84	26,837.28	1,688.09	< 0.0001
Error of measurement	32	272.79	8.52	-	-

t = 16.000, *P* = 0.004), but not at 48 h and 72 h (Table 15). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation. As shown, all had statistics significance at the level of time, molluscicide and concentration (Table 16).

Fish Toxicity: At a room temperature of 23–25°C, for fish exposed to 0.4 g/m^2 for 24 h, the mortality produced by LDS and WPN were 40% and 100% respectively. The LC_{50} results for 24 h exposure to LDS and WPN were 0.5657 mg/L and 0.2049 mg/L respectively (Table 17).

Cost Comparison: The costs of the two molluscicides were compared by assuming an immersion treatment of the same area in the field. For 10% LDS, 4 g/m^2 × 10,000 m^2 = 40,000 g (40 kg/hectare), 10 yuan/kg × 40kg = 400 yuan/hectare. For 50% WPN, 2 g/m^2 × 10,000 m^2 = 20,000 g (20 kg/hectare), 35 yuan/kg × 20 kg = 700 yuan/hectare. Thus, the treatment cost for LDS was less than that of WPN (Table 18).

DISCUSSION

While many molluscicides are effective in killing snails, there are problems regarding both toxicity to non-target animals and degradation (Zidan, 2002; Graebing et al., 2004b; Xu et al., 2006). Some molluscicides have been reported as being effective in killing snails and being less toxic to fish, such as the fruit of *Solanum xanthocarpum*, niclosamide- based chemicals (Wei et al., 2002), and polymeric niclosamide formulations against *Biomphalaria alexandrina* snails (Kenawy, 2004). The mixing of nictinalinate with niclosamide not only increase effectiveness in killing snails, but also reduces toxicity to fish (Cai et al., 2002; Yuan et al., 2005b, 2007; Xu et al., 2007; Dai et al., 2008).

Currently, results with LDS show that at concentrations of 0.4 mg/L, and 72 hour exposure by immersion, snail mortality values for LDS and WPN were 100% and 96.70% respectively in the lab, and 100% and 95.33% respectively in the

TABLE 11. Percent mortality for time and concentration of two drugs investigated by powdering tests in the lab.

	LDS			WPN		
Active Concentration (g/m^2)	1 d	3 d	7 d	1 d	3 d	7 d
0.05	18.67 ± 4.16	30.67 ± 5.77	38.00 ± 10.00	16.67 ± 4.16	23.33 ± 2.31	36.00 ± 10.00
0.1	50.67 ± 10.07	61.33 ± 1.15	71.33 ± 5.77	46.67 ± 10.07	58.00 ± 2.00	67.33 ± 2.31
0.2	64.00 ± 10.58	81.33 ± 1.15	88.67 ± 2.31	63.33 ± 11.55	74.00 ± 5.29	84.67 ± 5.77
0.4	80.00 ± 8.72	87.33 ± 2.31	91.33 ± 3.06	74.00 ± 10.39	81.33 ± 5.03	90.00 ± 5.29
0.8	84.67± 11.02	94.67 ± 1.15	98.00 ± 0.00	84.67 ± 5.77	92.00 ± 3.46	98.00 ± 0.00
1.6	90.67 ± 2.31	100.00 ± 0.00	100.00 ± 0.00	88.67 ± 5.77	97.33 ± 1.15	100.00 ± 0.00
3.2	96.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	94.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control	1.67 ± 0.58	3.33 ± 0.58	4.00 ± 0.00	1.67 ± 0.58	3.33 ± 0.58	4.00 ± 0.00

TABLE 12. Results of analysis of variance for two drugs investigated by powdering tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	4,421.06	2,210.53	103.38	< 0.0001
Molluscicide	1	205.44	205.44	5.38	0.0268
Concentration	7	15,4133.97	22,019.14	577.13	< 0.0001
Error of measurement	64	1,368.44	21.38	-	-

TABLE 13. Percent mortality for time and concentration of two drugs investigated by powdering tests in the field.

Active Concentration (mg/L)	LDS			WPN		
	1 d	3 d	7 d	1 d	3 d	7 d
0.2	47.40 ± 6.46	74.13 ± 4.63	84.93 ± 7.18	34.30 ± 4.36	58.87 ± 5.35	85.33 ± 3.52
0.4	65.00 ± 6.37	93.50 ± 3.77	99.33 ± 1.15	60.43 ± 5.61	82.07± 4.14	94.60 ± 1.42
0.8	83.07 ± 12.08	98.93 ± 1.01	100.00 ± 0.00	72.73 ± 6.03	91.53 ± 1.00	100.00 ± 0.00
Control	2.93 ± 0.25	3.97 ± 0.76	3.77 ± 0.45	3.33 ± 0.51	3.97± 0.76	4.07 ± 0.23

TABLE 14. Results of analysis of variance for two drugs investigated by powdering tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	13,624.06	6,812.03	799.09	< 0.0001
Molluscicide	1	429.25	429.25	27.00	< 0.0001
Concentration	3	80,511.84	26,837.28	1,688.09	< 0.0001
Error of measurement	32	272.79	8.52	-	-

TABLE 15. Percent mortality of snail eggs for time and concentration of two drugs investigated by immersion tests in the lab.

Active Concentration (mg/L)	LDS			WPN		
	24 h	48 h	72 h	24 h	48 h	72 h
0.05	9.00 ± 5.29	14.33 ± 5.13	29.67 ± 5.03	8.67 ± 5.51	13.33 ± 12.74	25.67 ± 3.06
0.1	14.00 ± 5.29	34.67 ± 0.58	52.67 ± 2.52	13.67 ± 5.51	28.33 ± 15.28	49.00 ± 5.29
0.2	33.00 ± 1.73	57.33 ± 2.08	80.67 ± 1.15	24.33 ± 5.13	52.67 ± 2.31	75.00 ± 4.00
0.4	63.67 ± 4.04	92.33 ± 2.08	94.00 ± 3.46	55.00 ± 0.00	82.33 ± 2.08	89.00 ± 1.73
0.8	75.00± 0.00	93.00 ± 2.65	96.33 ± 1.15	64.33 ± 1.15	90.67 ± 1.15	95.00 ± 0.00
1.6	85.00 ± 0.00	95.67 ± 0.58	100.00 ± 0.00	81.00 ± 1.73	95.00 ± 0.00	100.00 ± 0.00
3.2	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	85.67 ± 1.15	100.00 ± 0.00	100.00 ± 0.00
Control	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

TABLE 16. Results of analysis of variance for two drugs investigated by immersion tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	14,250.167	7,125.08	875.81	< 0.0001
Molluscicide	1	416.84	416.84	14.51	< 0.0001
Concentration	7	17,1157.44	24,451.06	851.09	< 0.0001
Error of measurement	64	520.67	8.14	-	-

field. We showed that the effect of using these two molluscicides is the same, but LDS had a significantly lower toxicity to fish. Moreover the cost of LDS is much lower than that of WPN. The cost of WPN is now 35 Chinese Yuan per kilogram while LDS is 10 Chinese Yuan per kilogram. In addition, as the production process for LDS is simpler than for WPN, production costs could be reduced by a factor approximately ten. Hence, production costs and application fees are both decreased. For example, the cost of 50% WPN is 35 Yuan/per kilogram and 10% LDS is 10 Yuan/per kilogram. As killing the snails by immersion method over the same area of one hectare, using 50% WPN with 2 mg/L needs 20 kg and 700 Chinese Yuan, while using 10% LDS with 4 mg/L needs 40 kg and 400 Chinese Yuan. It is clear that the cost of LDS is 42% lower than the cost of WPN.

TABLE 17. Fish toxicity LC$_{50}$ for LDS and WPN in the lab.

Name of Drug	Time (h)	LC$_{50}$ (mg/L)	95% Confidence Interval	
			Lower Limit	Upper Limit
LDS	8	1.7493	1.1411	3.5128
	24	0.5657	0.4091	0.7823
	48	0.4666	0.3391	0.6301
	72	0.4666	0.3391	0.6301
WPN	8	0.4820	0.3081	0.7448
	24	0.2049	-	-
	48	0.1381	0.0964	0.1968
	72	0.0851	0.06	0.1179

The molluscicidal efficacy of LDS was observed in different concentrations and different exposure times in the lab. The results show that snail mortality increased with both time and concentration. The effectiveness of LDS was clearly related to time of exposure.

The fish toxicity tests showed that for fish exposed to 0.4 g/m^2 for 24 h, the LC$_{50}$ of LDS was 1.7 times that of WPN (LDS 0.5657 mg/L; WPN 0.2049 mg/L). This means LDS is safer for the fish than WPN.

In our opinion, LDS is a reliable molluscicide that can be readily used in different habitats and at low cost. We recommend it as the molluscicide of choice for use in a variety of habitats.

TABLE 18. Comparing the cost between LDS and WPN.

Content	10% LDS	50% WPN	Gradient with LDS to WPN (%)
Original materials	abio-alkali	ethanol amine	
Price (RMB per yuan/per ton)	3,000	17,000	Decline 82.35
Commercial price (RMB yuan/per ton)	56,000	70,000	Decline 20.00
Packing materials	abio-salt	diatomite	
Price of packing (RMB yuan/per ton)	500	350	Augment 42.85
Price of commercial molluscicide (RMB yuan/per ton)	10,000	35,000	Decline 71.42
Commercial molluscicide			
Using dosage (kg/hectare)	40/1	70/1	
Market price (RMB yuan/hectare)	400/1	700/1	Decline 42.85

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REDESCRIPTION AND ANATOMY OF THE VIVIPAROUS FRESHWATER GASTROPOD *HEMISINUS LINEOLATUS* (W. WOOD, 1828) FROM JAMAICA (CERITHIOIDEA, THIARIDAE)

Maria I. Gomez[1]*, Ellen E. Strong[2] & Matthias Glaubrecht[1]

ABSTRACT

The West Indies, well known for its distinctive geological history, is well suited for studies in biogeography and biodiversity. However, the lack of comprehensive studies for much of its unique fauna has hampered insights into their systematics, evolution and phylogeography. The scarcity of data is particularly pronounced for freshwater gastropods in the viviparous family Thiaridae – represented in the archipelago by *Hemisinus* Swainson, 1840 and *Cubaedomus* Morrison, 1951. Hitherto described only on the basis of shell morphology, we here redescribe *Hemisinus lineolatus* (W. Wood, 1828), the type species of *Hemisinus*, based on type material and museum collections. The anatomy of this species is also described, representing the first such comprehensive study of any Neotropical thiarid. The synonymy of *Hemisinus buccinoides* Reeve, 1860, with *H. lineolatus* is confirmed. The species is found to possess a mixture of distinctly thiarid features (e.g., operculum, rachidian, midgut, brood pouch) and features previously undocumented in the family (e.g., gonochorism with ~equal sex ratios, glandular pallial oviduct with spermatophore bursa in lateral lamina, spermatophore organ, statoconia). Some of these features (e.g., statoconia, spermatophore organ) are also found in members of the apparent sister group to the Thiaridae – the African-Asian freshwater Paludomidae. Consequently, the combination of features in *Hemisinus* is consistent with a basal position in the family.

Key words: morphology, systematics, Paludomidae, brood pouch, Greater Antilles, Neotropics.

INTRODUCTION

Located within the Caribbean Plate, the islands of the West Indies have been considered a laboratory of biogeography and evolution (Ricklefs & Bermingham, 2008). The West Indian islands are sufficiently isolated to favor the presence of endemism in many groups of organisms, but they are also close enough to each other and to the mainland to allow a dynamic interaction with the surrounding continents (Buskirk, 1985; Woods, 1989; Iturralde-Vinent & MacPhee, 1999; Hedges, 2001, 2006; Woods & Sergile, 2001). The West Indian archipelago is composed of three groups of islands: the Greater Antilles, the Bahamas, and the Lesser Antilles. Jamaica, the third largest island of the Greater Antilles, is considered to be one Caribbean biogeographical region by itself, as result of its unique geology and faunal endemism (Bland, 1866; Buskirk, 1985; Iturralde-Vinent & MacPhee, 1999). On Jamaica, while the land snail fauna appears to be the most varied and unique among the whole West Indies archipelago (Bland, 1866; Goodfriend, 1986; Paul & Donovan, 2005; Rosenberg & Muratov, 2006), it seems to be the opposite for the freshwater representatives; the freshwater mollusks mainly belong to cosmopolitan genera, with exceptions only among the ampullariids (Ampullarioidea) and thiarids (Cerithioidea) (Simpson, 1894; Russell-Hunter, 1955).

Although freshwater cerithioideans are one of the richest gastropod components of freshwater faunas in the tropical areas of the world (Strong et al., 2008), they are still relatively poorly studied in the Neotropics. The generic

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[1] Museum für Naturkunde, Leibniz Institute for Research in Evolution and Biodiversity at the Humboldt University Berlin, Invalidenstraße 43, 10115 Berlin, Germany

[2] Smithsonian Institution, National Museum of Natural History, Department of Invertebrate Zoology, MRC 163, PO Box 37012, Washington, D.C. 20013-7012, U.S.A.

*Corresponding author: gomez.berning@googlemail.com

names *Aylacostoma* Spix, 1827, *Hemisinus* Swainson, 1840, and *Cubaedomus* Morrison, 1951, together with the subgenera *Semisinus* Morelet, 1849, *Verena* Moricand, 1841, *Longiverena* Spix, 1827, and *Basistoma* Lea, 1852, have been assigned to taxa inhabiting Central and/or South America (Chenu, 1859; Fischer, 1885; Thiele, 1928; Pilsbry & Olsson, 1935; Morrison, 1951, 1954; Jaeckel, 1969; Nuttall, 1990). Among these taxa, the limits between them still remain to be established. As a consequence, the distributional range for such genera as *Hemisinus*, which was originally allocated to Jamaica and Cuba, progressively expanded since the mid 19th century to include Central America, Peru, Venezuela, Surinam, Brazil and the Paraguay River (Reeve, 1860; Brot, 1862; Martens, 1873; Fischer, 1885; Ihering, 1901, 1909; Vernhout, 1914; Hylton-Scott, 1954; Simone, 2006). The same applies to those fossil shells that have been assigned to the Thiaridae due to superficial resemblance (e.g. Nuttall, 1990).

Knowledge of the Jamaican Thiaridae, based solely on 19th century shell descriptions made by such naturalists as Adams (1845, 1849, 1851), Chitty (1853), Bland (1861, 1866), and Vendryes (1899), has hardly increased since. Unfortunately, these studies failed to record precise localities. From Jamaica, *Hemisinus lineolatus* (W. Wood, 1828) and *H. buccinoides* Reeve, 1860, have been described based on the morphology of single or few shells. In order to shed light on the history of the genus *Hemisinus*, this paper aims to use geographic data, in an integrative approach, on occurrences, shell morphology and the soft body anatomy to resolve the taxonomy and systematics of these enigmatic natives of the West Indies.

MATERIALS AND METHODS

The reviewed collections are composed mainly of specimens obtained on Jamaica's easily accessible areas, such as Montego Bay or Bog Walk, whereas the eastern territories have been poorly sampled (Fig. 2). Embryonic shells were measured from scanning electron micrographs. Adult shells were measured with calipers with a precision of 0.1 mm (n = 874). Measurements include shell height and width, aperture length and width, length of the last whorl, length of the last three whorls, and whorl number. After exploratory statistical analysis (data not shown), 44 juvenile shells were excluded from subsequent analyses to diminish the number of outliers. In order to compare type material for the two Jamaican nominal taxa to each other and to other Antillean samples, multivariate and graphical statistic analyses were performed. Because the variables mentioned above are in different units, all variables were normalized by dividing the raw values by their standard deviations (data not shown). A Principal Components Analysis (PCA) was performed using Palaeontological Statistics version 1.68 (Hammer et al., 2001). The two most explanatory variables identified via PCA, were compared graphically (scatterplot, boxplot) in SPSS 15.0 for Windows.

Samples preserved in 70% ethanol were used for anatomy, histology, radulae extraction, and SEM observation of embryonic shells. Remarkable is the scarcity of sufficient and adequately preserved soft body samples. The anatomical studies here presented are based on the examination of 30 female and male specimens of *H. lineolatus* from the ANSP and ZMB collections. The anatomy was studied using a Leica stereo microscope MZ 9.5 with camera lucida. Midgut anatomy is based on three specimens (ANSP 12088A, n= 1; ANSP 12087I, n = 2), and was studied by cutting around the periphery of the midgut chamber along the posterior, right and anterior margins and deflecting the roof to the left. Complete male and female bodies, as well as isolated male and female pallial gonoducts, were studied histologically (ANSP 12087I, n = 4). Two females (ANSP 12087H) containing embryonic shells in the brood pouch were decalcified in successive solutions of 7% nitric acid (HNO_3; for three days), sodium sulphate (Na_2SO_4; for one day) and distilled water (one day) according to Romeis (1989). Specimens were dehydrated and paraffin embedded using an automatic Shandon Hypercenter XP 167506S. Tissues were sectioned at 12 µm on a Leica SM 2000R microtome, stained with haematoxylin/eosin and preserved with Canada balsam. Histological serial sections were photographed with a microscanning Jenoptik Prog/Res 3012.

The radula was extracted and cleaned as described by Holznagel (1998). Cusp/denticle formula is as follows: (1) rachidian (number of left side cusps/median denticle(s)/number of right side cusps), (2) lateral teeth (inner cusps/pronounced denticle/outer cusps), (3) marginal teeth (number of cusps on inner marginal tooth + number of cusps on outer marginal tooth).

Museum Abbreviations

ANSP	Academy of Natural Sciences, Philadelphia, Pennsylvania
BMNH	The Natural History Museum, London, U.K.
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts
USNM	National Museum of Natural History, Smithsonian Institution, Washington, D.C.
ZMB	Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University, Berlin, Germany

RESULTS

Hemisinus Swainson, 1840

Hemisinus Swainson, 1840: 341
Tania Gray, 1840: 148 [not available: *nomen nudum*]; not made available by Gray (1842: 61, 90), or by H. Adams & A. Adams, 1854: 302.
Semisinus P. Fischer, 1885: 701 [invalid: unjustified emendation of *Hemisinus*].
Type Species: *Strombus lineolatus* W. Wood, 1828, by monotypy.

Remarks

In his *Supplement* to the *Index Testaceologicus*, Wood (1828: 13–14, pl. 4) illustrated some shells from Asia and the Antilles under the generic allocation of *Strombus*, with specific names based on the most characteristic attribute of their shells. On a later page of this work (1828: 42) and in a subsequent paper (Wood, 1829: 31) indicated that Lamarck would place some of these species in his freshwater genus *Melania* Lamarck, 1799. In order to clearly delimit groups, Swainson (1840) erected *Hemisinus* for neotropical shells characterized by a "*melanid*" shape, but with the base of the aperture contracted and emarginated, in addition to a crenated outer lip. Fischer (1885) suggested *Semisinus* (*semi* = half, *sinus* = notch) as an emendation of *Hemisinus*, with Johnson & Fox (1891) returning to the use of *Hemisinus*. *Tania* Gray, 1840, was a *nomen nudum* when first proposed, and later sunk by Gray himself into the synonymy of *Hemisinus* (Gray, 1847: 153). *Haemisinus* was an incorrect subsequent spelling by Rotarides (1933) used for a Pleistocene fossil from Hungary (and an incorrect usage for species of *Fagotia* Bourguignat 1884), and therefore has no standing in nomenclature.

Distribution

Greater Antilles (Jamaica and Cuba), Central America, Lesser Antilles (Barbados), and South America (Fig. 1). Nuttall (1990) also assigned fossil shells from the Paleogene and Neogene of Colombia, Ecuador and Peru to this genus.

Hemisinus lineolatus (W. Wood, 1828)

Strombus lineolatus W. Wood, 1828: 13, pl. 4, fig. 11.
Synonym: *Hemisinus buccinoides* Reeve, 1860: pl. 1, fig. 3a, b.

Other references

Melania lineolata – W. Wood, 1829: 31; Gray in Griffith & Pidgeon, 1833: pl. 13, fig. 4 [*non Melania lineolata* Gray in Griffith & Pidgeon, 1833: pl. 14, fig. 4]; Gray, 1847: 153; Philippi, 1848: 33, pl. 5, fig. 10; Jousseaume, 1889: 233; Cossmann, 1909: 150; Morrison, 1954: 376; Vega & Perrilliat, 1992: 604.
Hemisinus lineolata – Swainson, 1840: 341.
Melanopsis lineolata – C. B. Adams, 1849: 45; 1851: 187; Hanley, 1854–1858: pl. IV, fig. 29; Bland, 1861: 24; Orcutt, 1928: 12.
Melanopsis lineata – Poey, 1851: 399; 1856: 3
Hemisinus lineolatus – Gray, 1857: 103; H. Adams & A. Adams, 1854: 302, pl. 32, fig. 2a, b; Chenu, 1859: 291, fig. 1995; Reeve, 1860: plate 1, fig. 4a, b; Brot, 1878: 373, pl. 38, fig. 6, 6a–e; Kobelt, 1882: 131; Johnson & Fox, 1891: 34; Henderson, 1894: 33; Ihering, 1901: 672; Ihering, 1909: 311; Vernhout, 1914: 36; Baker, 1930: 30; Pain, 1956: 103; Nuttall, 1990: 239, figs. 224–226; Glaubrecht, 1996: 185.
Hemisinus (*Basistoma*) *lineolatus* – Brot, 1862: 61.
Melania (*Hemisinus*) *lineolata* – Martens, 1873: 51, 60.
Semisinus lineolatus – Fischer, 1885: 702.
Semisinus lineolata – Vendryes, 1899: 13.
Melanopsis lineolatus – Vendryes, 1899: 13.
Hemisinus (*Hemisinus*) *lineolatus* – Thiele, 1928: 401; 1929: 201; Wenz, 1939: 718, fig. 2075; Jaeckel, 1969: 814, pl. 3, fig. 70.
Aylacostoma (*Hemisinus*) *lineolatum* – Morrison, 1951: 9; 1954: 376.

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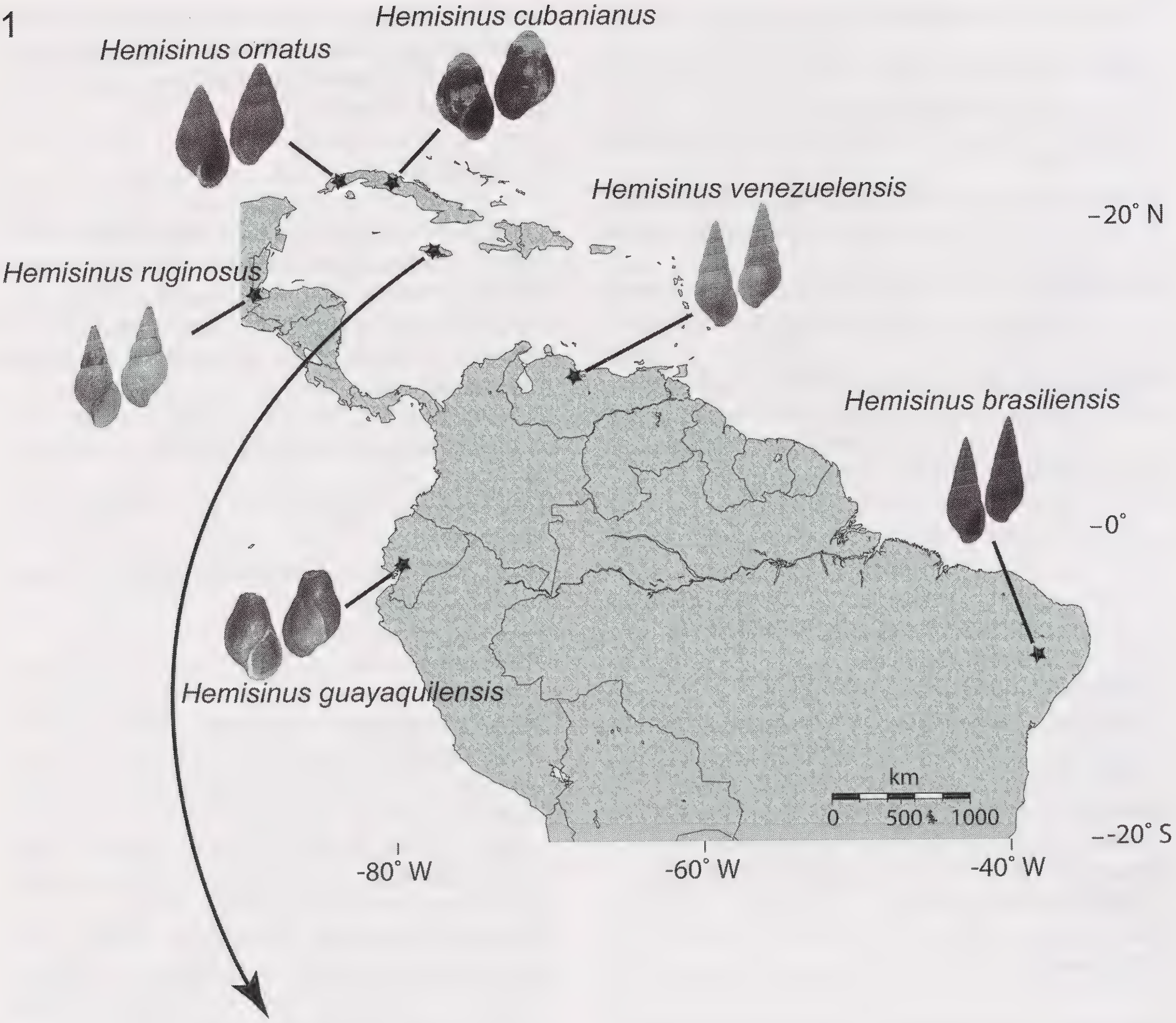

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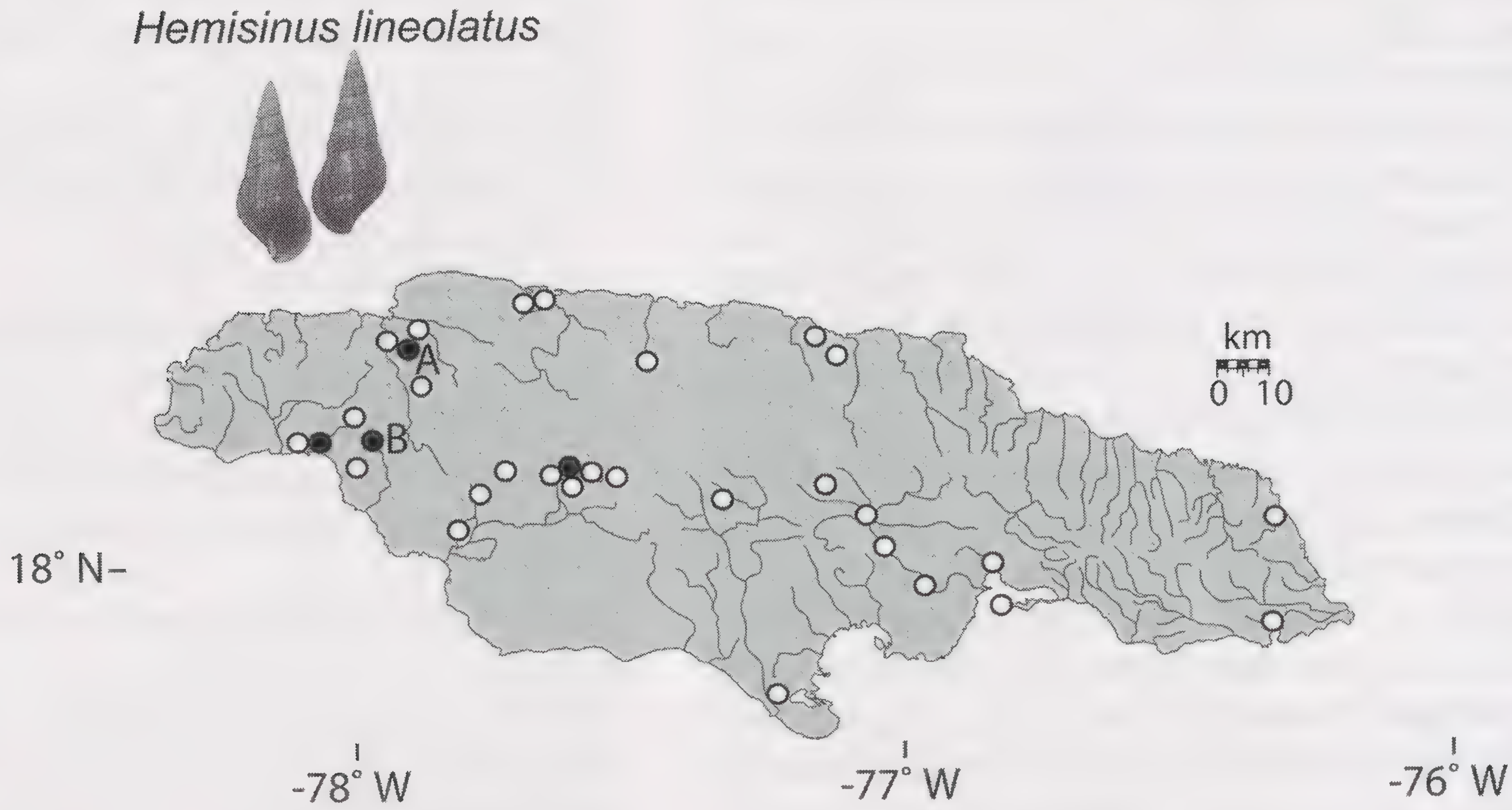
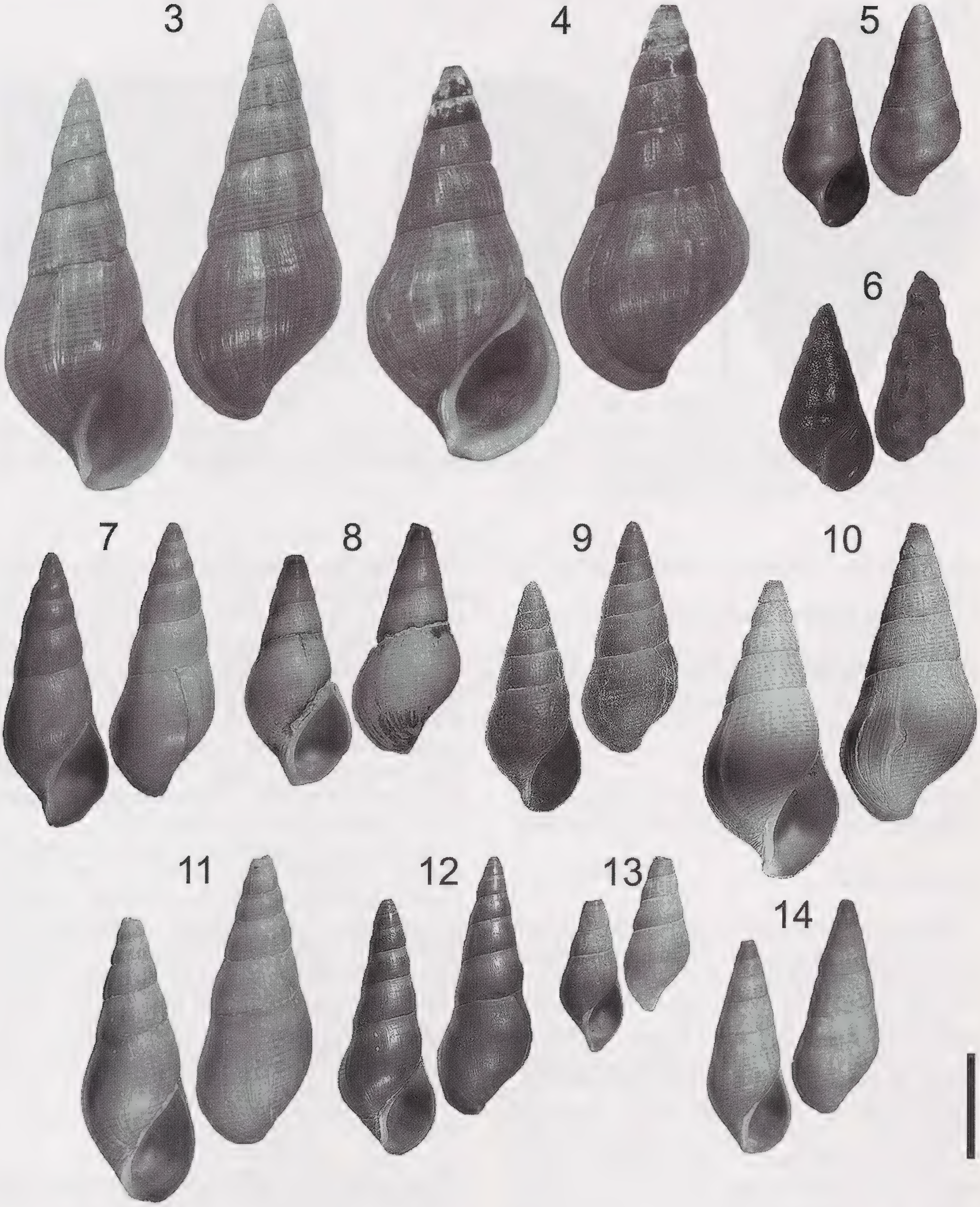
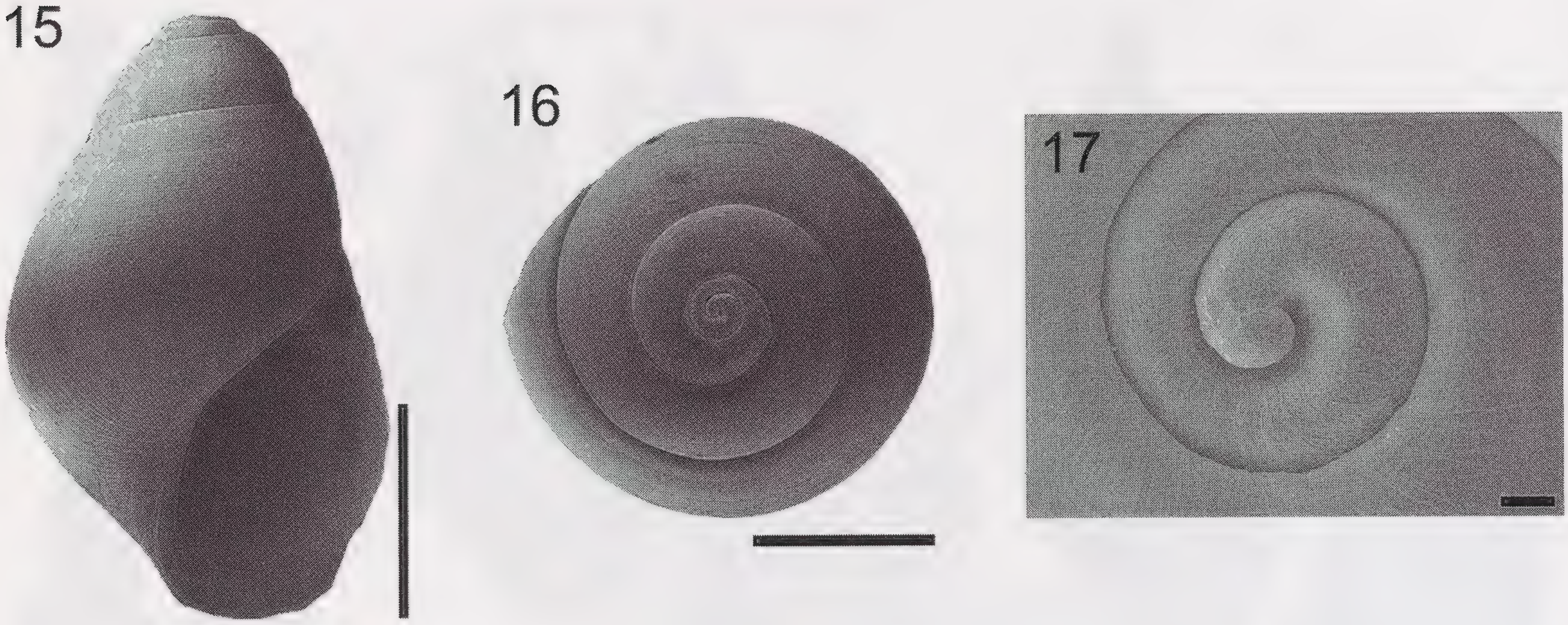


FIGS. 1, 2. Distribution of *Hemisinus* Swainson, 1840. Not all nominal taxa shown. FIG. 1: *Hemisinus* spp.; FIG. 2: Occurrence of the type species *Hemisinus lineolatus* (W. Wood, 1828) on Jamaica; see text for details. ○ = museum shell material; ● = alcohol preserved material examined here for anatomy and histology; A, Anchovy Gully (ANSP 12087H-I); B, Black River, St. Elizabeth parish (ZMB 107126).



FIGS. 3–14. *Hemisinus* spp. FIG. 3: Lectotype of *Hemisinus lineolatus* (W. Wood, 1828) (BMNH 1984206/1); FIG. 4: Lectotype of *Hemisinus buccinoides* Reeve, 1860 (BMNH 1984208); FIGS. 5–14: Museum specimens of *Hemisinus lineolatus* from Jamaica; shells arranged from west to east. FIG. 5: Westmoreland Parrish (USNM 127820); FIG. 6: St. James Parrish (ANSP 12087I); FIG. 7: St. Elizabeth Parrish (USNM 397352); FIG. 8: Trelawny Parrish (USNM 395818); FIG. 9: Manchester Parrish (USNM 345479); FIG. 10: St. Ann Parrish (USNM 395800); FIG. 11: Clarendon Parrish (USNM 395751); FIG. 12: St. Catherine Parrish (USNM 453971); FIG. 13: St. Andrew Parrish (USNM 378057); FIG. 14: Portland Parrish (USNM 712035). Scale bar = 1 cm.



FIGS. 15–17. Embryonic shell of *Hemisinus lineolatus* from brood pouch (ANSP 12087H). FIG. 15: Apertural view. Scale bar = 300 µm; FIG. 16: Apical view. Scale bar = 200 µm; FIG. 17: Initial whorl of embryonic shell, apical view. Scale bar = 50 µm.

Aylacostoma lineolata – Simone, 2006: 82, pl. 199.
Hemisinus (*Basistoma*) *buccinoides* – Brot, 1862: 61.
Hemisinus lineolatus var. *buccinoides* – Kobelt, 1882: 131.

Type Locality

Strombus lineolatus: none given in the original description. *Hemisinus buccinoides*: Jamaica.

Type Material

Strombus lineolatus: Lectotype (Fig. 3) and six paralectotypes BMNH 1984206/1-7 Original label: front "*lineolatus* Wood, Hab. ?; *H. lineolata*; W. Sup. t. 4, f. 11; g. a. k., t. 13, f. 14; Holotype"; back "*Hemisinus lineolatus* Wood, Type, Hab. ?, Gray Collection". Additional label: "BMNH ZD 1984206, Lectotype of *Strombus lineolatus* Wood 1828 [Type species of *Hemisinus* Swainson 1840], Hab. ? [Caribbean], Described and figd Nuttall, C.P. 1990. *Bull. Br. Mus. Nat. Hist.*, (Geol.) 45 (2). Pp. 238–240, figs. 224a, b. PTO. Wood 1828: pl. 4 [Strombus fig 11]- - p. 42 [*Melania lineolata*], see Nuttall 1990 for full synonymy".

The original description is silent with respect to the number of specimens examined and does not contain a (holo)type designation. The height of a single shell was provided (h = 1½ inches [3.81 cm]). A lectotype (Fig. 3) was designated by Nuttall (1990) on the basis that it was the largest specimen and matched the size provided in the original description. In contrast to Nuttall's (1990: 240) statement that this sample is composed of "*four unlocalized Recent shells*", we found seven specimens (Lectotype and six paralectotypes). The discrepancy in reported numbers of specimens herein and in Nuttall is explained by an accompanying label stating: "*Thought to be a lot of 4 shells, 3 smaller shells later found under cotton wool – CP Nuttall May 1995*".

Hemisinus buccinoides (Fig. 4): Lectotype BMNH 1984208. Original label: "*Hemisinus* [Type of *buccinoides*] *buccinoides* Rve. Jamaica (?), Jamaica, M. C.; Holotype". Additional label "BMNH ZD 1984 208 Holotype of *Hemisinus buccinoides* Reeve 1860 = *H. lineolatus* (Wood 1828), Described and figd Nuttall, C.P. 1990. *Bull. Br. Mus. Nat. Hist.*, (Geol.) 45 (2). Pp 239–240, fig 226; Reeve Conch Icon pl. 1, fig. 3a, b)".

Remarks

The authorship of *Strombus lineolatus* has been the subject of several discussions. The species name has been attributed to Gray, to Griffith in Cuvier, to Gray in Griffith & Pidgeon, to Wood in Gray, or to Gray in Wood. Nevertheless, as confirmed by Petit & Coan (2008), the name was validly established by William Wood (1774–1857), who copied it from labels prepared by J. E. Gray in the British Museum.

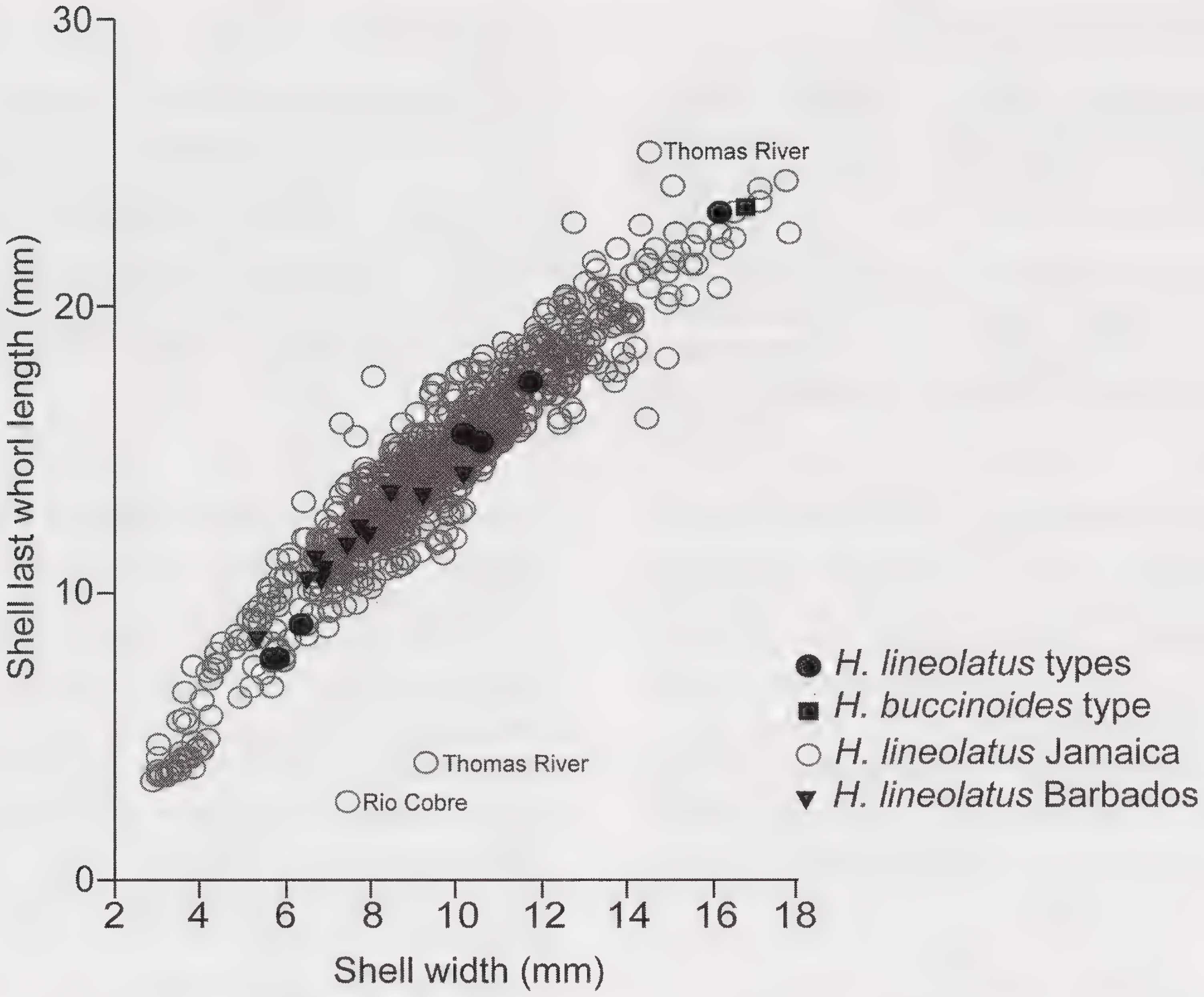


FIG. 18. Scatterplot of Jamaican *Hemisinus* shell width and last whorl length (outliers indicated). ● = types of *H. lineolatus* (BMNH 1984206/1-7); ■ = lectotype of *H. buccinoides* (BMNH 1984208); ○ = other Jamaican material examined; ▼ = *H. lineolatus* from Barbados (USNM 272287).

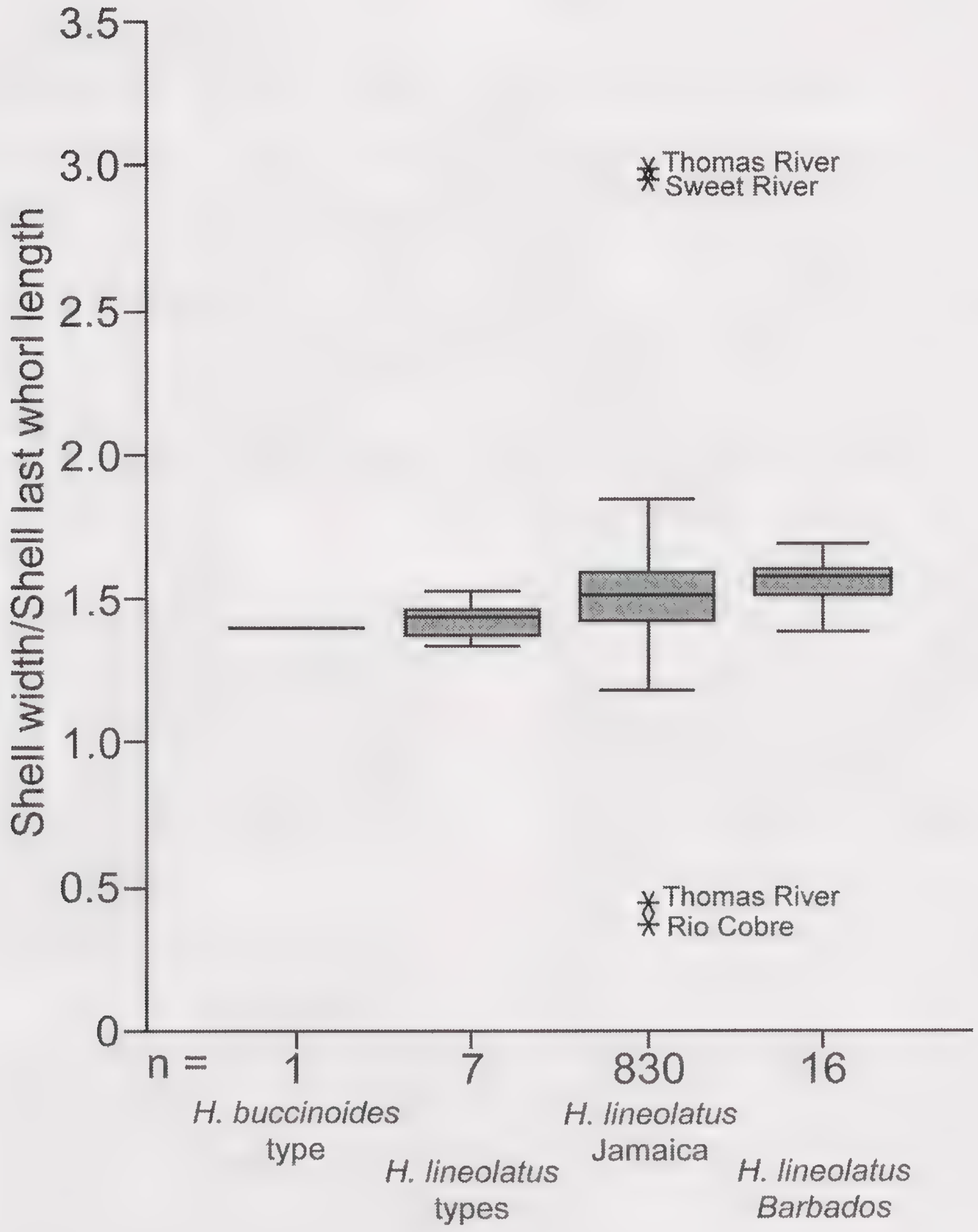


FIG. 19. Boxplot comparative analysis of shell width and last whorl length for *Hemisinus* shells from Jamaica (outliers indicated).

Additional Material Examined

Jamaica: (ANSP 26797, 26800, 122992, 123029; BMNH 1845.9.16.63, 1857.12.1.1135, 36, 37, 39, 41, 20070080, 20070085; ZMB 113036, 113037, 113038, n = 70). Westmoreland: Savanna-La-Mar (USNM 792463, n = 3); Ditch near Savanna-La-Mar (USNM 127820, n = 2; Fig. 5); Sweet River: near Savanna-La-Mar (USNM 127799, n = 8); Water Wheel (ANSP A12088A, A12088B; MCZ 195360, n = 10); Mackfield: Williamsfield Cave (BMNH 29.1.1907, n = 24); Mt. Pleasant (USNM 127821, n = 1). St. James: Anchovy Gully (ANSP A12087H, A12087I, n = 25; Fig. 6); Great River (USNM 127827, n = 2); Great River, first falls from the mouth (ANSP 153283, 159740; ZMB 113039, n = 7); Great River, seven miles south of Montego Bay (USNM 792464, n = 3); Montpellier (USNM 168599, 453972, n = 4); Spring Mount: (ANSP 160299, n = 25), Montego Bay: Mt. Horibb (MCZ 88922, n = 4). St. Elizabeth: Route A-2 about 2 mi. NE Middle Quarters (ANSP 375045, n = 11); Ipswich (USNM 168598, 453973, n = 11); Accompong River, Hole Cockpit (USNM 376369, n = 17); 3 miles north of Balaclava, Opfor Cave (USNM 374547, 396156, n = 9); Wallingford River (USNM 210911, n = 2); Balaclava (ANSP 226265, n = 2); Near Balaclava: Cave at sink of One Eye River (USNM 397352, 397082, 427066, 427096, n = 78; Fig. 7); Harbor Shore, Black River (USNM 427037, n = 1); Black River (MCZ 172867, n = 12). Trelawny: Falmouth: Bush Cay (USNM 395818, n = 8; Fig. 8); Near Westwood High School (USNM 399377, n = 1); Dornach River (ANSP 157713, n = 19). Manchester: One Eye River (USNM 374377, 375429, 375479, 398576, n = 62; Fig. 9); Middlesex, Black River north of Oxford (ZMB 107126, n = 5); Oxford Cave: top of hill over Oxford Cave, 3 mile north (USNM 398577, n = 50). St. Ann: Rio Bueno, near Dornach Power House (USNM 395800, 427097, n = 13; Fig. 10); St. Ann's Bay: Roaring River (ANSP 157714; USNM 127826, 453968, n = 46); Fern Gully (ANSP 160259; USNM 511960, 526273, n = 51). Clarendon: Frankfield (USNM 400639, n = 25); Rio Minho (USNM 395751, n = 11; Fig. 11); Portland (MCZ 195361, n = 6). St. Catherine: 1 mile from Ewarton to Moneague (USNM 396044, n = 1); Bog Walk (ANSP 61889; BMNH 20070081; USNM 127351, 127822, 453971, 394750, 394861, 395486, 395529, 427094, 427095, n = 111; Fig. 12); Bog Walk: Thomas River (USNM 427093, n = 31); Rio Cobre (ANSP 124742, 157271, 26798; USNM 700721, n = 79); Spanish Town: Canal drift (USNM 395743, n = 5). St. Andrew: Port Royal (USNM 442331, n = 1); Hunts Bay (USNM 378057, n = 1; Fig. 13). Portland: Priestmans River (USNM 712035, n = 9; Fig. 14). St. Thomas: Morant Bay (MCZ 115072, n = 8) (Fig. 2). Lesser Antilles: Barbados (USNM 272287, n = 16).

Distribution

The first time that *Hemisinus lineolatus* was reported from Jamaica was in Philippi (1848), wherein a shell that fits the illustration in Wood's original description is depicted. Afterwards, Poey (1851, 1856) restricted the species to the island, a view which is subsequently followed (Bland, 1861; Arango y Molina, 1865; Brot, 1878; Johnson & Fox, 1891; Henderson, 1894; Vendryes, 1899; Orcutt, 1928; Nuttall, 1990; Glaubrecht, 1996; Townsend & Newell, 2006). However, according to statistical analyses here performed (PCA, Scatter- and Box-plot), shells from Barbados (Lesser Antilles) are not different from those from Jamaica (Figs. 18, 19). Thus, until suitable material for anatomical studies of the Barbados specimens is available, the species distribution cannot be restricted to Jamaica.

On the other hand, *H. lineolatus* has been also reported in British Guiana and Surinam (Pain, 1956). Its presence, argued by Pain as man-made introduction, is not supported by Nuttall (1990), because the distribution data is both suspect and anomalous. Molecular analyses of specimens from continental America similar in shell shape and appearance with *H. lineolatus* showed that they are genetically distinct (Gomez, unpubl. data).

TABLE 1. Shell measurements (in millimeters) of embryonic shells (n = 7) obtained from the brood pouch of *Hemisinus lineolatus* from Jamaica. Abbreviations: dp, protoconch diameter; he, embryonic shell height; SD, standard deviation; we, embryonic shell width.

H. lineolatus	he	we	dp
range	2.42–4.88	2.18–3.17	0.29–0.74
mean	3.61	2.51	0.46
SD	0.90	0.40	0.15

Description

Shell: *Embryonic Shell*. Globose, smooth, faint axial and spiral lines, suture shallow, two to three whorls (Fig. 15). Color yellow or light brown. Apical cap smooth to only very weakly wrinkled, 1.25 whorls (Figs. 16, 17). Ranging in height from 2.4–4.8, mean = 3.61, standard deviation (SD) = 0.9 (n = 7) (Table 1).

Adult Shell. Conic, thin but solid, usually with apex not eroded, comprising five to six whorls. Spire with flattened whorls; body whorl inflated. Suture narrow and subsutural depression shallow, both forming colored band. Shell smooth, with faint axial and spiral growth lines. Color from yellow-brown to black, with reddish, spiral, interrupted color bands. Aperture ovate, angled above, expanded below, inside white (Figs. 3–14).

Maximum shell height, 42.7 mm, minimum shell height, 4.3 mm, mean = 22.3 mm, SD = 6.1 mm (n = 830) (Table 2). First two principal components explaining 93.137% of variance (Table 3). PC1 having high loading values for shell width and last whorl length; PC2 having high loading value for whorl number (Table 3).

Scatterplot and boxplot comparisons showing no significant morphometric differences among Jamaican populations nor between Jamaican and Barbados populations (Figs. 18, 19).

External Anatomy. Operculum (Fig. 20) ovate, corneous, reddish-brown; paucispiral, with small subterminal nucleus of about two whorls. Animal pale yellow, with brown or dark grey patches on tentacles, snout and anterior part of foot (Fig. 21). In females, brood pouch pore present on right side of head-foot, close to tip of pallial oviduct (Fig. 21). Snout short and broad. Tentacles broad, tapering, shorter than snout when retracted. Mantle edge with

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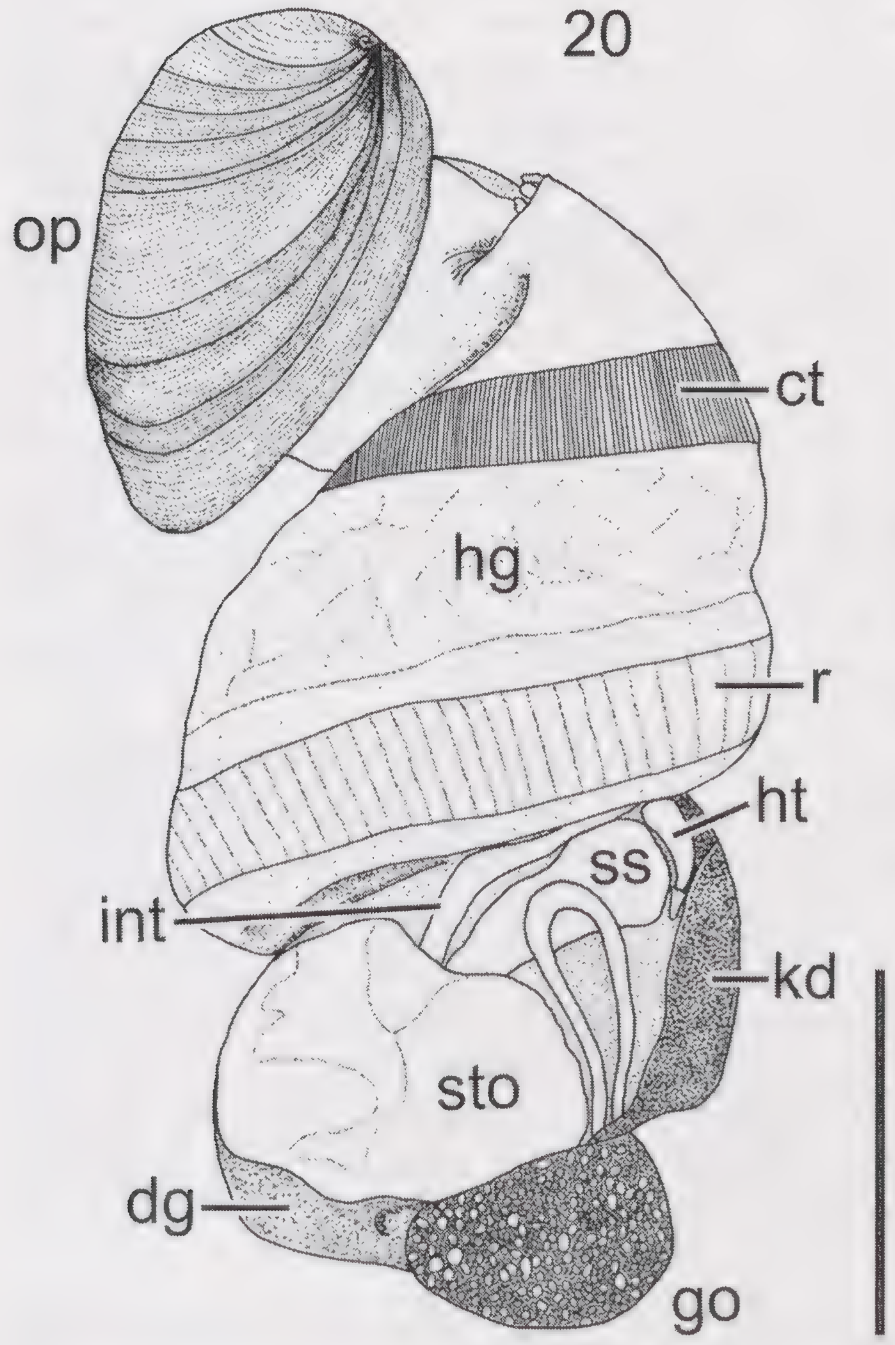

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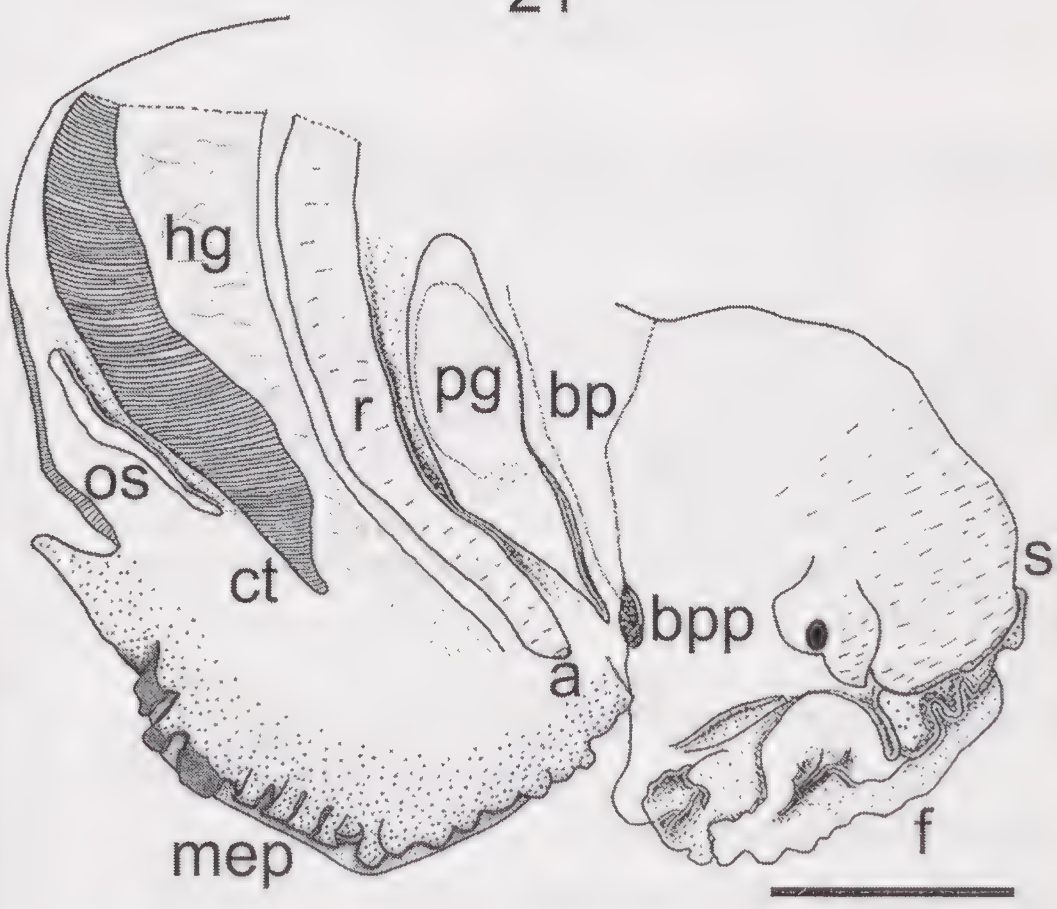


FIGS. 20, 21. Anatomy of *Hemisinus lineolatus* (Westmoreland, Jamaica, ANSP 12088A-B). FIG. 20: External anatomy, ventral view. Scale bar = 5 mm; FIG. 21: Anatomy of mantle cavity and brood pouch. Scale bar = 2 mm. Abbreviations: a, anus; bp, brood pouch; bpp, brood pouch pore; ct, ctenidium; dg, digestive gland; f, foot; go, gonad; hg, hypobranchial gland; ht, heart; int, intestine; kd, kidney; mep, mantle edge papillae; op, operculum; os, osphradium; pg, pallial gonoduct; r, rectum; sn, snout; ss, style sac; sto, stomach.

TABLE 2. Shell measurements of type specimens of *Hemisinus lineolatus* (lectotype BMNH 1984208) and *H. buccinoides* (lectotype BMNH 1984206/1), and Jamaican specimens of *H. lineolatus* from museum collections (n = 830). Abbreviations: h, shell height; la, aperture height; ltw, last three whorls; lwl, last whorl length; SD, standard deviation; w, shell width; wa, aperture width; wn, whorl number.

Species	h	w	la	wa	lwl	ltw	wn
H. lineolatus							
lectotype	39.78	16.19	16.50	8.20	23.31	32.30	9
H. buccinoides							
lectotype	37.17	16.78	17.91	8.45	23.43	31.69	6
H. lineolatus Jamaica							
range	4.35–42.77	2.90–14.56	2.75–17.02	1.34–7.47	3.46–25.40	4.35–36.06	3–6
mean	22.35	9.42	10.14	4.20	14.20	19.20	5.39
SD	6.10	2.60	2.82	1.58	3.76	5.38	1.31

13–21 papillae (n = 17; mean = 17; SD = 2.01) (Fig. 21). Mantle cavity extending approximately one whorl. Hypobranchial gland well developed, thick, with transverse grooves (Fig. 20). Ctenidium straight, long, with attached base of leaflets, narrow anteriorly and broad posteriorly (Fig. 21). Osphradium conspicuous, near anterior end of ctenidium, slightly curved, short, about one-third of ctenidium in length (Fig. 21). In some specimens (Black River, ZMB 107126, n = 5), osphradium slightly more than twice average length.

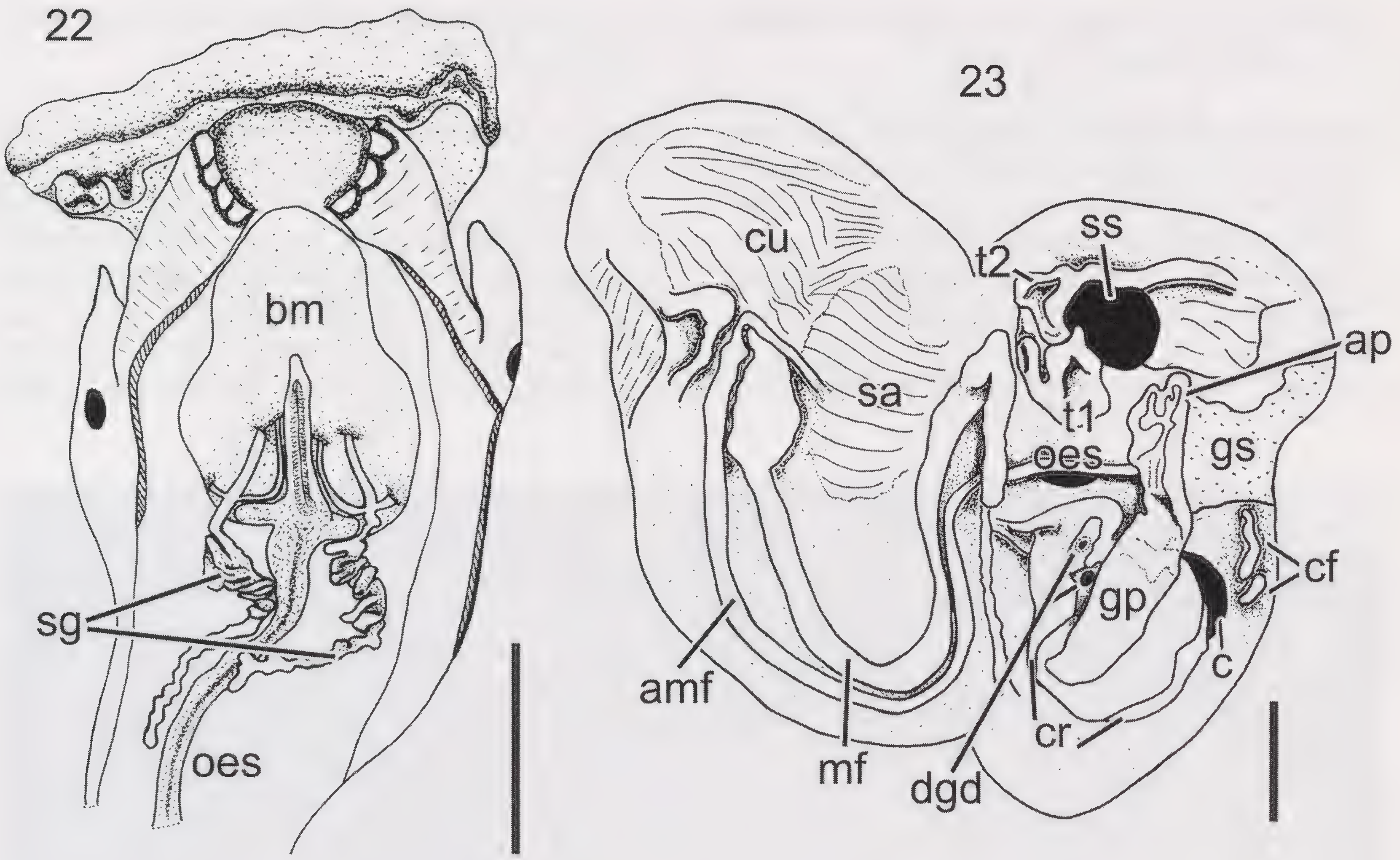
Alimentary System: Foregut. Buccal mass robust, pear-shaped (Fig. 22). Radular sac short, slightly curving behind, reaching base of anterior esophagus (Fig. 22). Radula with 140 rows (average of n = 5). Rachidian (Figs. 24–27) pentagonal, approximately twice broader than high, with concave anterior edge, v-shaped base. Cutting edge bearing rectangular, rounded central cusp, with two to three rounded (Fig. 26) or pointed (Fig. 27) denticles on either side (2–3/1/2–3). Rachidian with basal denticle at each outer corner. Lateral teeth bearing broad, squarish central cusp flanked by two to three short, rounded to pointed cusps on either side (2–3/1/2–3) (Figs. 26, 27). Marginal teeth (Figs. 28, 29) long, spatulate, with narrow flanges along shaft. Broad, rounded cutting edge bearing four to five short rounded denticles (Fig. 28), or up to eight slender, fringe-like denticles (Fig. 29).

TABLE 3. Principal component analysis on the correlation matrix of *Hemisinus lineolatus* on seven shell parameters (n = 830). Abbreviations: h, shell height; la, aperture height; ltw, last three whorls; lwl, last whorl length; PC, principal component; w, shell width; wa, aperture width; wn, whorl number.

				Component loadings	
PC	Eigen value	Variance (in %)	Data	PC 1	PC 2
1	5.51109	78.73	h	-0.4133	0.1335
2	1.00851	14.407	w	-0.4135	-0.1138
3	0.23881	3.4116	la	-0.4124	-0.1115
4	0.13618	1.9455	wa	-0.3849	-0.1634
5	0.04719	0.6741	lwl	-0.4176	-0.0644
6	0.04038	0.5769	ltw	-0.3934	0.0696
7	0.01784	0.2549	wn	-0.1046	0.9597

Thick buccal retractors inserting laterally near middle of buccal mass, extending to lateral walls of cephalic hemocoel adjacent to cerebral ganglia. Salivary glands (Fig. 22) opening dorso-laterally to buccal cavity. Glands long, tubular, passing through nerve ring, extending short distance posteriorly; left salivary gland passing above supra-esophageal ganglion. Mid-esophageal gland absent.

Midgut (Fig. 23). Esophagus opening to midgut floor at left. Stomach roof with large, oval sorting area and small, irregularly folded cuticularized anterior portion. Marginal fold with broad U-shaped anterior tip; fold extending from major typhlosole, alongside sorting area, around posterior end of stomach. Accessory marginal fold emerging from esophageal



FIGS. 22, 23. Digestive system of *Hemisinus lineolatus*. FIG. 22: Foregut morphology, dorsal view (Black River, Jamaica; ZMB 107126). Scale bar = 2 mm; FIG. 23: Midgut morphology, dorsal view (Westmoreland, Jamaica; ANSP A12088A). Scale bar = 1 mm. Abbreviations: amf, accesory marginal fold; ap, accessory pad; bm, buccal mass; c, caecum; cf, caecal folds; cr, crescentic ridge; cu, cuticle; dgd, digestive gland duct; gp, glandular pad; gs, gastric shield; mf, maginal fold; oes, esophagus; sa, sorting area; sg, salivary glands; ss, style sac; t1, major typhlosole; t2, minor typhlosole.

aperture, paralleling marginal fold around posterior end of stomach. Crystalline style pocket rounded and small. Gastric shield narrow. Glandular pad large, rounded, more than twice as long as broad. Large, textured accessory pad present at anterior end of glandular pad at left. Crescentic ridge emerging behind esophageal aperture, extending posteriorly around glandular pad, fusing to glandular pad above caecum behind gastric shield; caecum shallow, extending short distance under glandular pad. Two caecal folds opposite caecum; posterior fold half size of anterior fold. Paired digestive gland ducts opening to crescentic groove at left of glandular pad. Style sac and intestinal groove completely separated by fused typhlosoles.

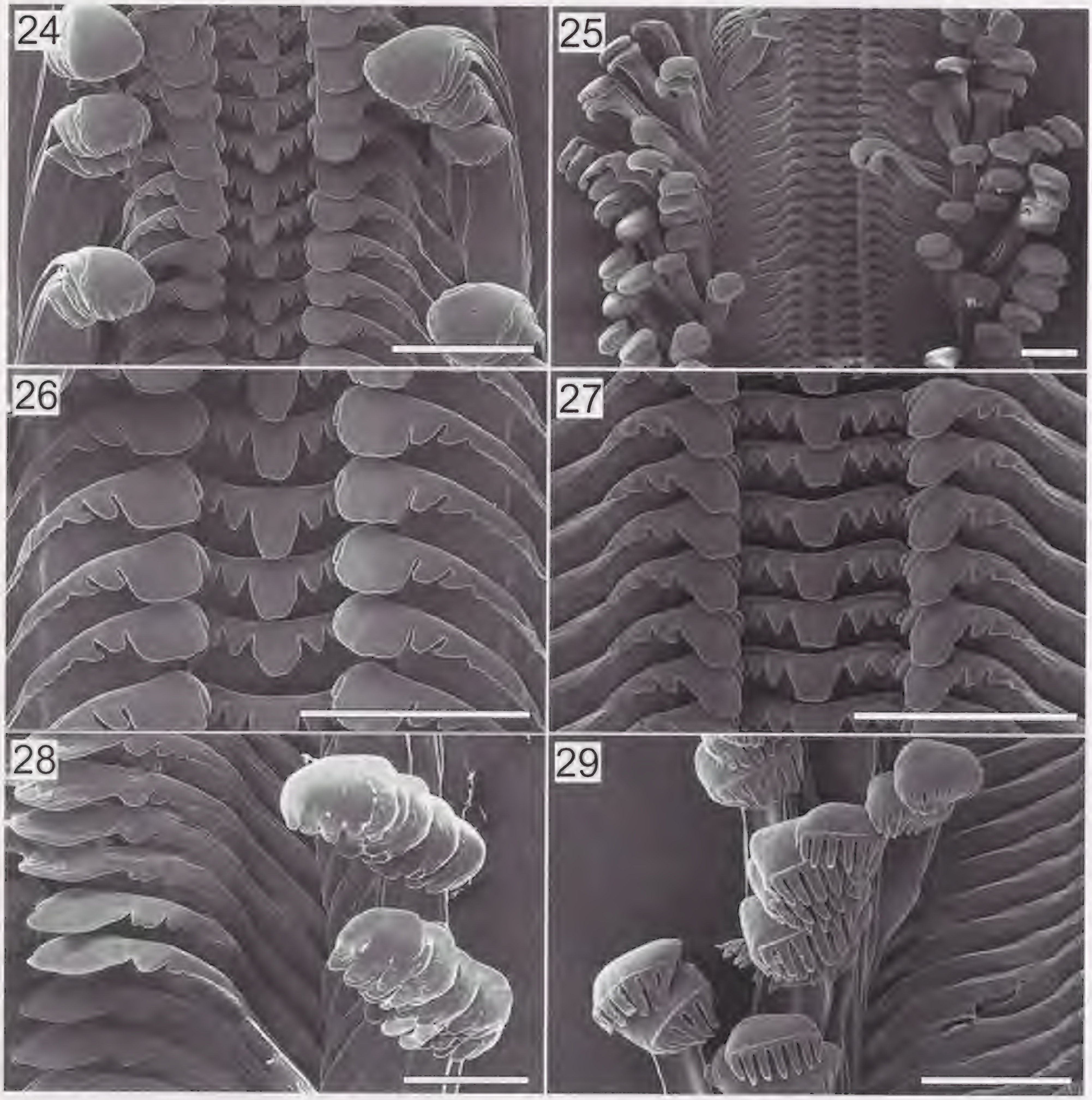
Hindgut. Proximal intestine passing around anterior tip of style sac, forming U-curve in front of gastric chamber partially overlying style sac (Fig. 20), continuing anteriorly alongside kidney, entering pallial roof above pallial gonoduct, terminating with simple anus near mantle edge (Fig. 21).

Reno-Pericardial System. Kidney large, wide, reaching base of mantle cavity between intestine and ctenidium, bounded behind by digestive gland and style sac (Fig. 20). Lumen subdivided into chambers. Pericardial coelom deep, narrow, extending posteriorly under kidney along style sac.

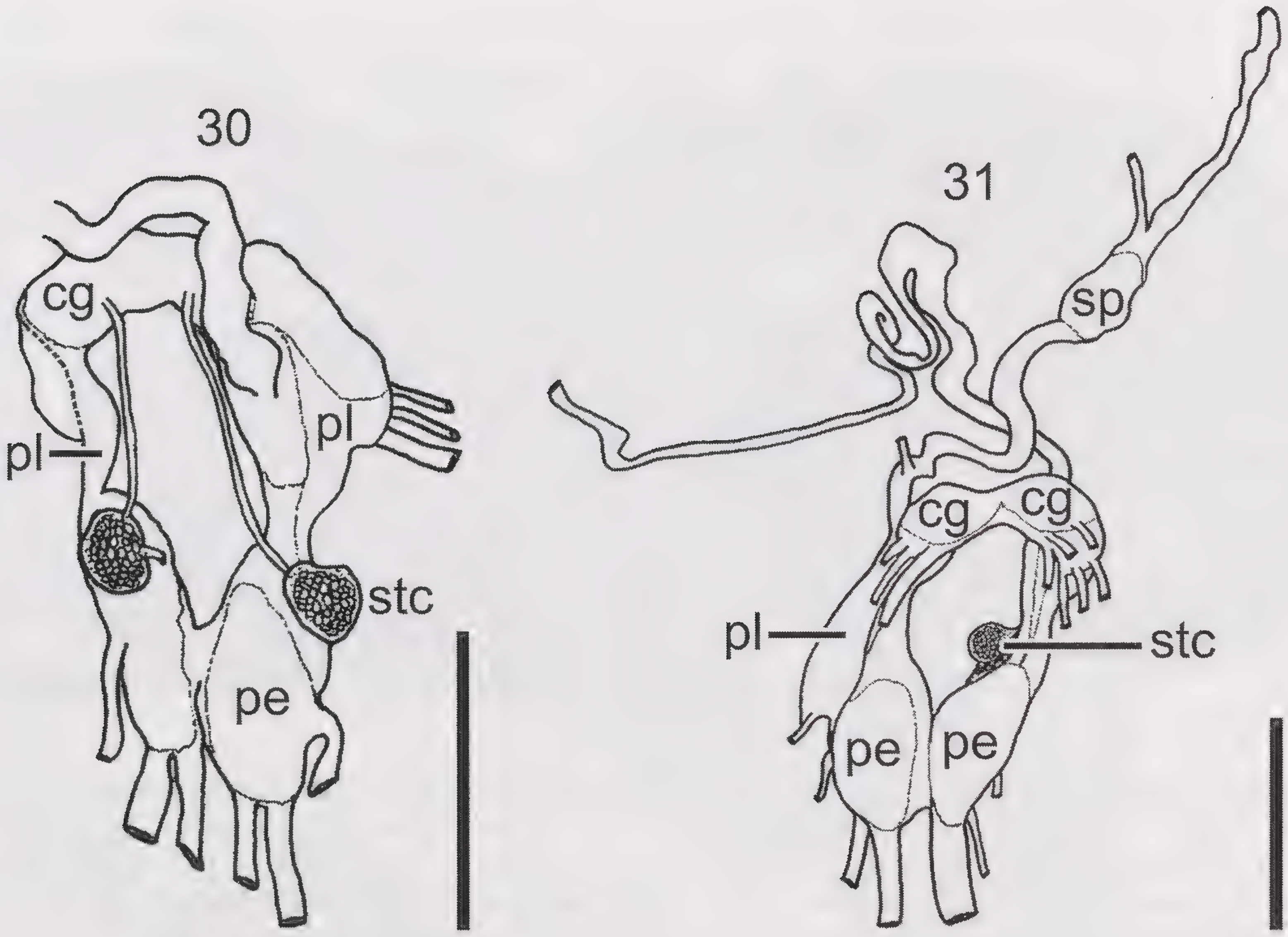
Nervous System (Figs. 30, 31): Circum-esophageal nerve ring lying immediately behind buccal mass. Cerebral ganglia above esophagus, connected by short commissure; each ganglion producing six nerves. Pleural ganglia behind and below cerebral ganglia, forming almost one mass on each side (Fig. 30). Two pedal ganglia united by short commissure, lying ventrally in foot, with three prominent accessory nerves (Fig. 31). Sub-esophageal ganglion closely adjacent to left pleural ganglion. Dialyneurous connection between right pleural and sub-esophageal ganglion (not shown). Long connective joining supra-esophageal ganglion and right pleural ganglion (Fig. 31). Statocysts with up to 50 angular statoconia, present dorsally and behind pedal ganglia, with long connectives to cerebral ganglia (Fig. 30).

Reproductive System: Gonad (Fig. 20) developing from tip of visceral whorls to posterior end of stomach, overlying digestive gland; similar in general aspect in males and females. Visceral gonoduct emerging ventrally in both sexes. Sexual dimorphism determined by presence of brood pouch pore at right side of adult females' head-foot (Fig. 21). Gender ratio of 52% females and 48% males (n = 30).

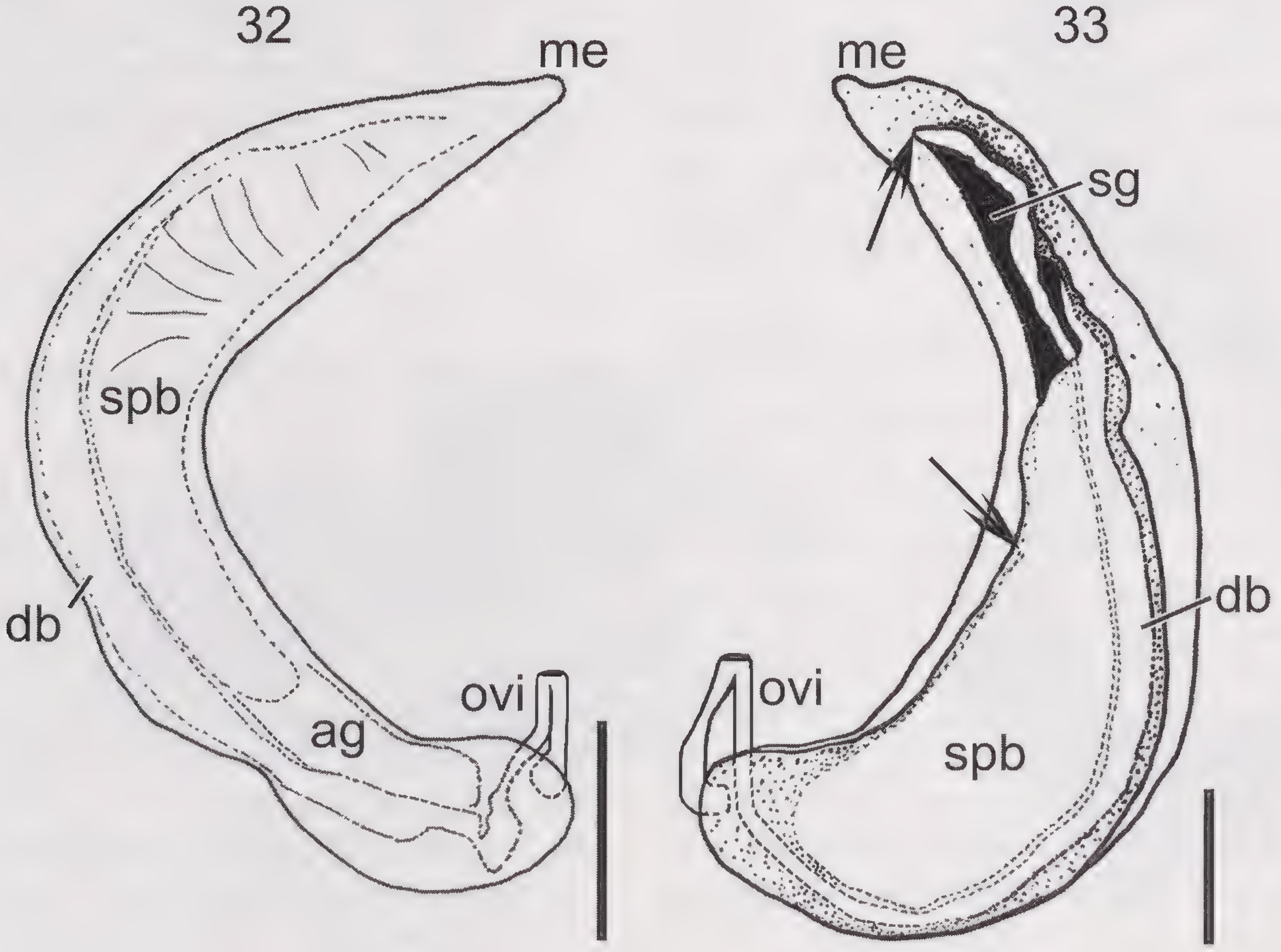
Female Reproductive System: Renal oviduct short, curved, thick (Fig. 32: ovi), with two branches; one branch entering pallial gonoduct posteriorly, second dorsal branch embedded in lateral lamina entering more anteriorly (Fig. 32). Pallial oviduct opening to mantle cavity through ventral slit along anterior half (Fig. 33: arrows). Pallial oviduct with



FIGS. 24–29. Radula of *Hemisinus lineolatus* from Jamaica. FIGS. 24, 25: Overview of radular ribbon. FIG. 24: ANSP 120781; FIG. 25: ZMB 107126. Scale bar = 100 µm; FIGS. 26, 27: Detail of rachidian and lateral teeth. FIG. 26: ANSP 120781; FIG. 27: ZMB 107126. Scale bar = 100 µm; FIGS. 28, 29: Detail of lateral and marginal teeth. FIG. 28: ANSP 120781. Scale bar = 50 µm; FIG. 29: ZMB 107126. Scale bar = 100 µm.



FIGS. 30, 31. Circum-esophageal nerve ring of *Hemisinus lineolatus*. FIG. 30: Circum-esophageal nerve ring, rear view (Westmoreland, Jamaica; ANSP 12088A); FIG. 31: Nerve ring, frontal view (Westmoreland, Jamaica; ANSP 12088A). Scale bars = 1 mm. Abbreviations: cg, cerebral ganglia; pe, pedal ganglia; pl, pleural ganglia; sp, supra-esophageal ganglion; stc, statocyst.



FIGS. 32, 33. Female pallial oviduct of *Hemisinus lineolatus* (Anchovy Gully, Jamaica, ANSP 12087H). FIG. 32: External, right lateral view of lateral lamina; FIG. 33: Internal view of lateral lamina (external wall, i.e. medial lamina, of albumen gland removed), arrows indicate extent of gonoduct opening to mantle cavity. Scale bars = 1 mm. Abbreviations: ag, albumen gland; db, dorsal branch of renal oviduct; me, mantle edge; ovi, renal oviduct; sg, sperm gutter; spb, spermatophore bursa.

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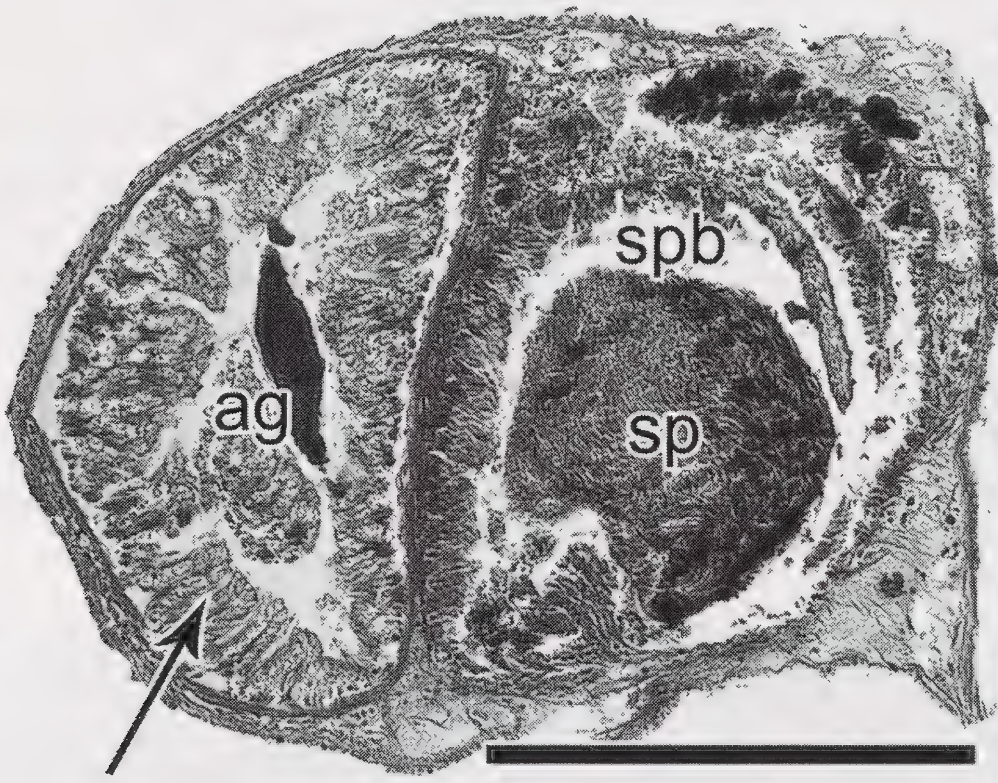

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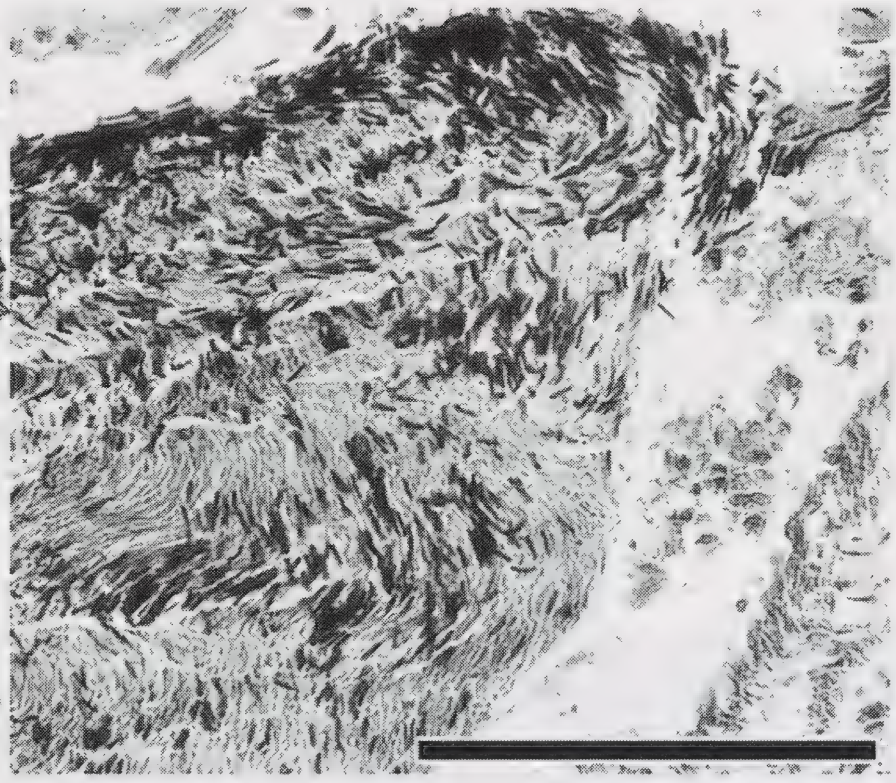


FIGS. 34, 35. Histological detail of a female pallial oviduct of *Hemisinus lineolatus* (Anchovy Gully, Jamaica; ANSP 12087I; dorsal is uppermost). FIG. 34: Albumen gland with glandular epithelium (arrow) and spermatophore bursa with unorientated sperm. Scale bar = 0.5 µm; FIG. 35: Detail of sperm in lumen of spermatophore bursa. Note darkly staining sperm heads. Scale bar = 0.125 µm. Abbreviations: ag, albumen gland; sp, sperm; spb, spermatophore bursa.

unspecialized glandular epithelium anteriorly, and forming highly folded albumen gland posteriorly lined with tall, basophilic, prismatic cells (Fig. 34: arrow). Sperm gutter in lateral lamina beginning slightly back from anterior end of pallial oviduct, deepening posteriorly, enclosing entrance to spermatophore bursa at midpoint of gonoduct (Fig. 33). Gutter receiving dorsal branch of renal oviduct near entrance of bursa. Lumen of spermatophore bursa containing densely packed unorientated sperm (Figs. 34, 35).

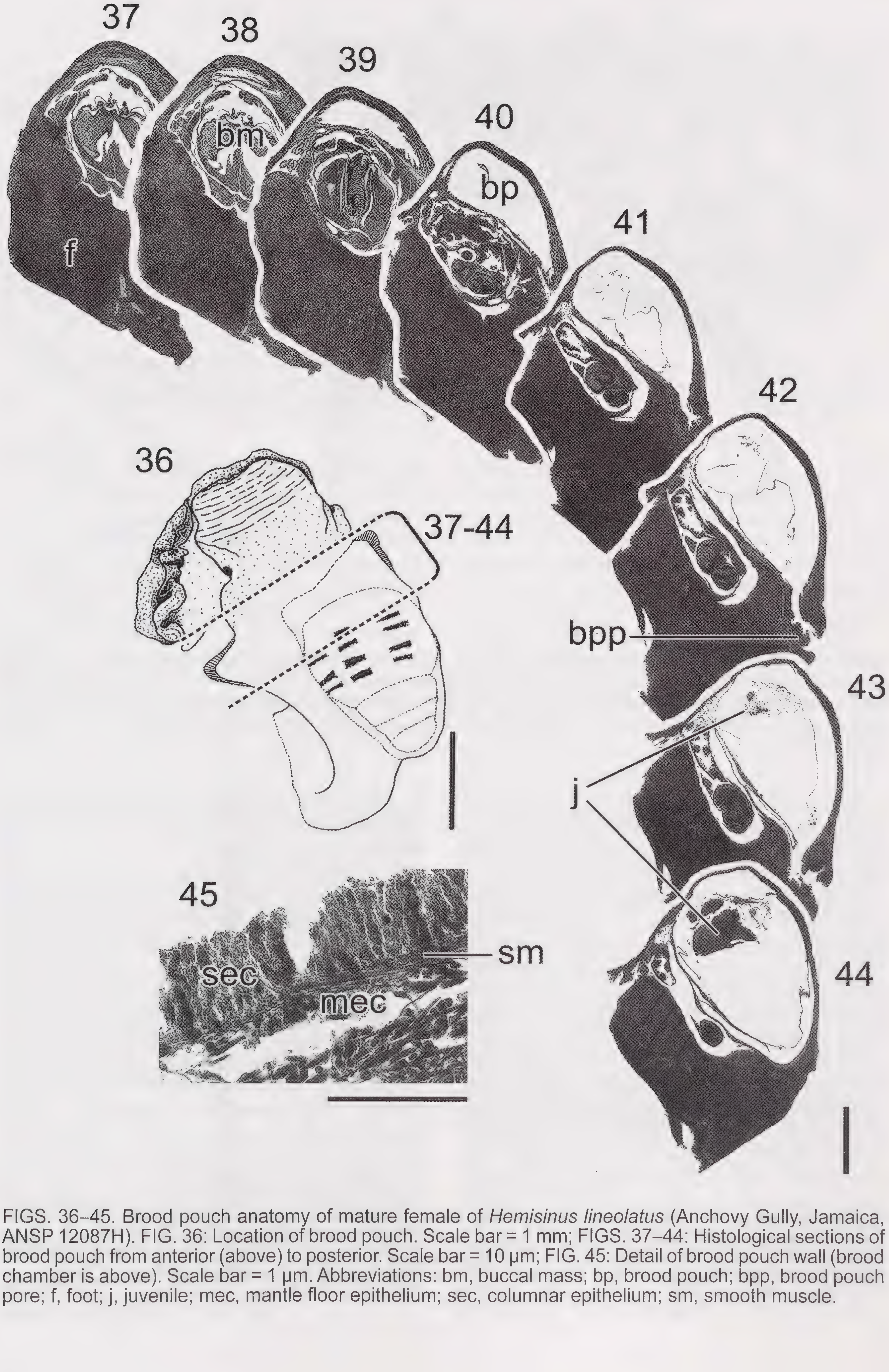
Females with cephalic brood pouch extending posteriorly from brood pore on side of neck behind right eye to end of mantle cavity (Fig. 36). Lumen of brood pouch dorsal, above esophagus; lumen not compartmentalized (Figs. 37–44). Wall of brood pouch (Fig. 45) formed by thin layer of smooth muscle lined by thin layer of tall columnar cells with large, basal nuclei (Fig. 45). Brood pouch containing single large, shelled juvenile (ANSP 12087I, 12087H, Anchovy Gully; n = 10), or up to three juveniles of variable size (ZMB 107126, Black River; n = 5). Embryonic shells surrounded by thin epithelial tissue. No eggs or early developmental stages present.

Male Reproductive System: Narrow vas deferens straight, entering posterior end of prostate near base of mantle cavity. Prostate opening to mantle cavity along entire length except short, fused segment at base of mantle cavity (Figs. 46–56). Mid-dorsal portion of prostate forming short, glandular spermatophore forming organ. Organ terminating blindly posteriorly, communicating anteriorly with prostate lumen (Figs. 50–52). Organ lumen lined with tall prismatic epithelium (Fig. 57), containing unorientated sperm (Fig. 58: arrow).

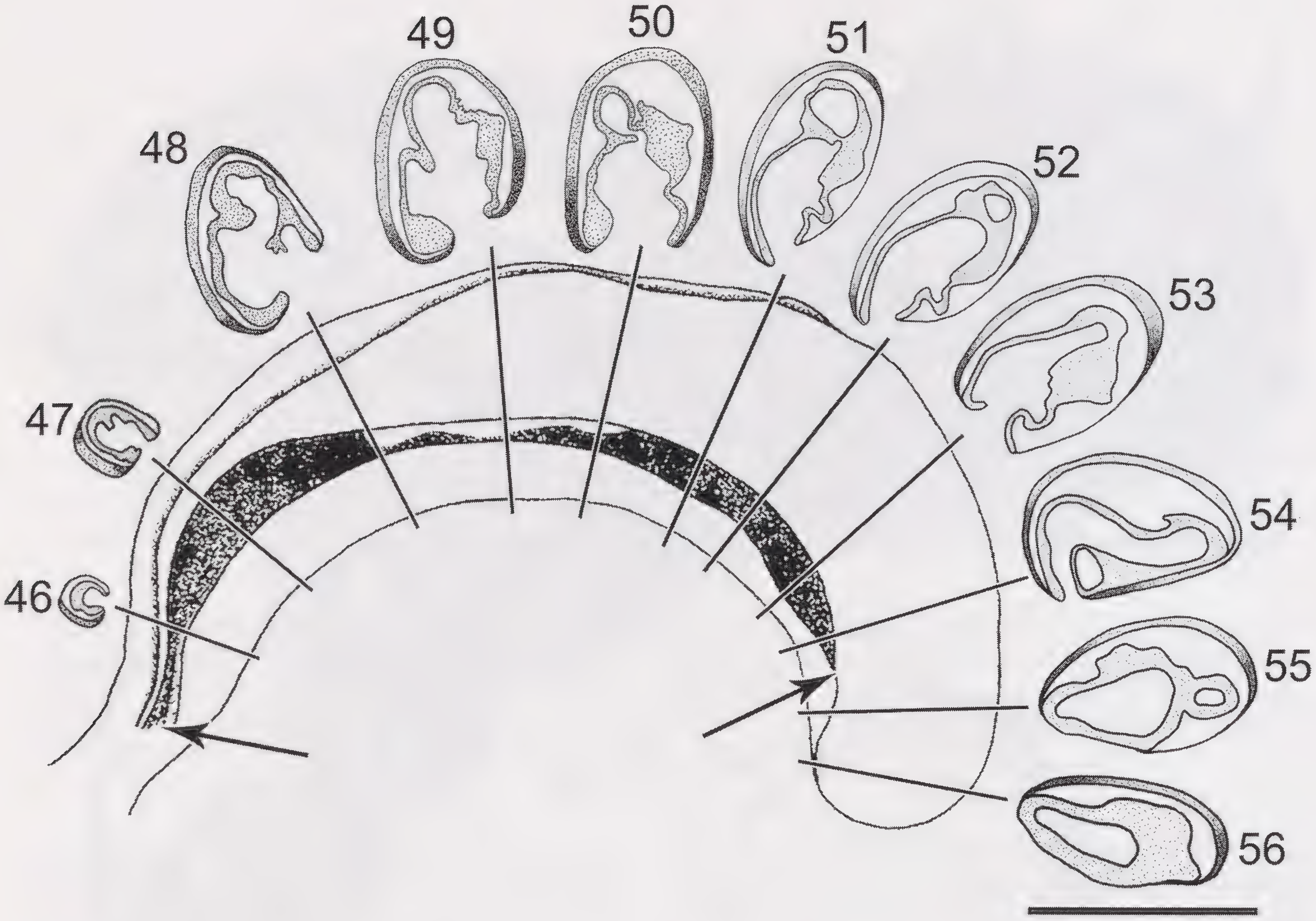
DISCUSSION

Intraspecific Variability in *Hemisinus lineolatus*

Hemisinus buccinoides was described by Reeve (1860: pl. 1, fig. 3a, b) for an unknown number of shells from Cuming's collection, the origin of which was thought to be Jamaican (Reeve, 1860). It was assumed by Brot (1878) and Simpson (1894) that *buccinoides* was a synonym of *lineolatus*. Shortly thereafter, the species name *buccinoides* disappeared from the literature, leaving only the name *lineolatus* for Jamaican Thiaridae. Comparative analyses of *Hemisinus* shell width and last whorl length through west-east and north-south gradients in Jamaica reveal neither patterns nor trends (data not shown). The lack of significant differences between shells of *H. lineolatus* and *H. buccinoides*, as demonstrated by graphical statistical analysis (Figs. 18, 19), supports this hypothesis.



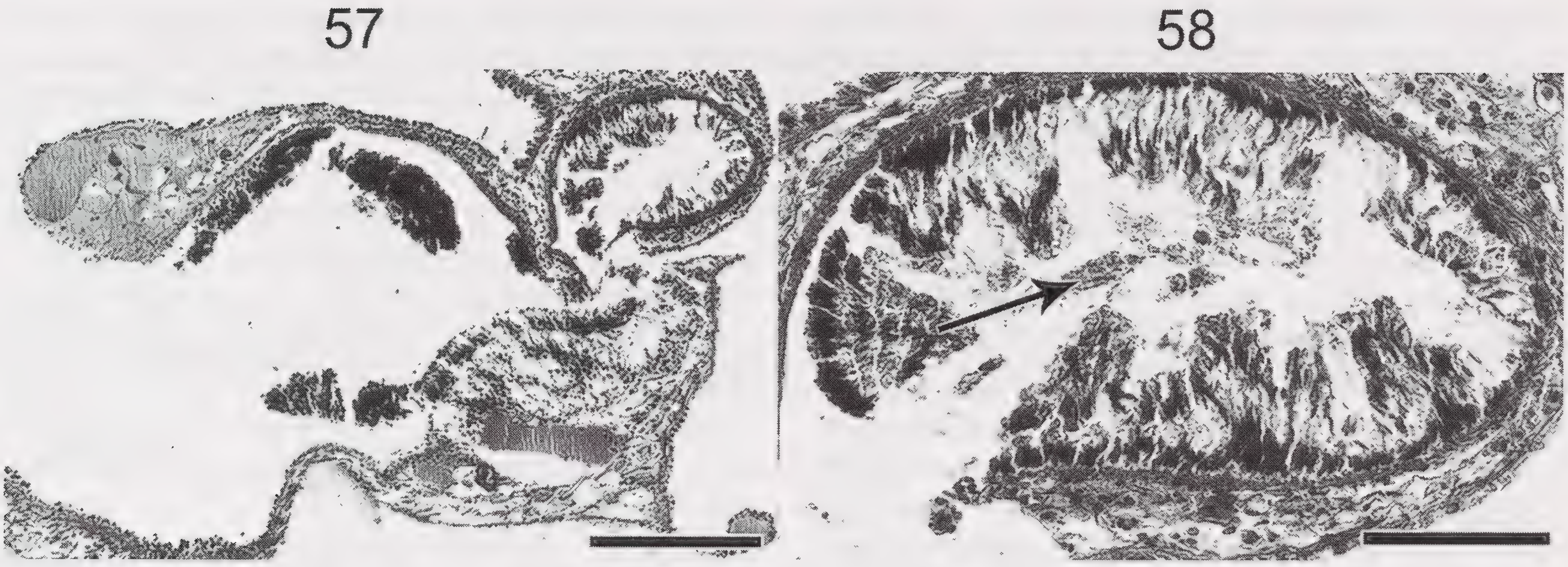
FIGS. 36–45. Brood pouch anatomy of mature female of *Hemisinus lineolatus* (Anchovy Gully, Jamaica, ANSP 12087H). FIG. 36: Location of brood pouch. Scale bar = 1 mm; FIGS. 37–44: Histological sections of brood pouch from anterior (above) to posterior. Scale bar = 10 µm; FIG. 45: Detail of brood pouch wall (brood chamber is above). Scale bar = 1 µm. Abbreviations: bm, buccal mass; bp, brood pouch; bpp, brood pouch pore; f, foot; j, juvenile; mec, mantle floor epithelium; sec, columnar epithelium; sm, smooth muscle.



FIGS. 46–56. Male pallial gonoduct of *Hemisinus lineolatus* (Anchovy Gully, Jamaica; ANSP 12087I). Reconstruction of the internal anatomy with sections from anterior (left) to posterior. Arrows indicate extent of opening to mantle cavity. FIGS. 47–49: Main chamber with longitudinal folds building grooves; FIGS. 50–52: Spermatophore forming organ; FIG. 55: Vas deferens entrance to the prostate. Scale bars = 1 mm.

However, some anatomical differences were found between one population of the northwest Jamaican coast (Anchovy Gully, St. James parish, ANSP 12087I, H; n = 10) and one from the central part of the island (Black River, St. Elizabeth parish, ZMB 107126; n = 5) in the shape of radular teeth, osphradium length, length of the oviductal opening to the mantle cavity, and number of juveniles inside the brood pouch. Specimens from Anchovy-Gully show rounded radular denticles, an oviductal aperture that extends to the middle of the pallial gonoduct, and only one embryo in each brood pouch. Specimens from the Black River have pointed and slender radular denticles; the osphradium is twice as long as that of the Anchovy-Gully specimens; the oviductal aperture extends slightly beyond the middle of the gonoduct; and at least three juveniles of different size were found inside the brood pouch. However, adult and embryonic shell morphology, as well as anatomy of the nervous system, male pallial gonoduct, and stomach showed no differences between the populations.

In gastropods, radular morphology and environmental conditions are highly correlated. Transfer experiments with Neotropical Littorinidae showed radular changes within 40 days (Andrade & Solferini, 2006). In freshwater taxa, differences in radular morphology, distinguished by the shape and relative size of the denticulation, are present both within species flocks in ancient lakes (Rintelen et al., 2004) and between partly isolated populations (Hunter, 1975; Evans, 1989). Even food deprivation events lead to modifications in radular secretion after 120 days (Smith & Russel-Hunter, 1990). Such differences can be attributed to ecophenotypic plasticity, which does not support hypotheses of geographic race formation or incipient speciation (Hunter, 1975; Evans, 1989; Smith & Russel-Hunter, 1990; Andrade & Solferini, 2006).



FIGS. 57, 58. Histology of male pallial gonoduct of *Hemisinus lineolatus* (Anchovy Gully, Jamaica; ANSP 120871). FIG. 57: Cross section of prostate at level of spermatophore forming organ (see Fig. 50); FIG. 58: Detail of spermatophore forming organ (arrow indicates sperm). Scale bars = 0.25 µm.

Similarly, significant variation in fecundity within species has been found between populations of tropical viviparid snails determined by such factors as periphyton quantity and quality, population density, current velocity, and physio-chemical variables, including water temperature, dissolved oxygen, and calcium concentrations (New South Wales, Department of Primary Industries, 2007). For other freshwater snails, Dillon (2000) also established that higher current velocities (as caused by rainfall) could be responsible for lower survivorship and fecundity. Searching for environmental factors that could correlate with brood differences between Jamaican populations, it was found that specimens from Anchovy-Gully were sampled during the dry season (July), whereas the Black River specimens were collected during the peak of the rainy season (October). Further sampling will be necessary to establish if the observed differences are a consequence of seasonality.

Consequently, the above-mentioned differences between populations are interpreted as representing intraspecific variation. It can be concluded that *Hemisinus* is represented in Jamaica by only one native species.

Reproductive Biology

Based on the fact that thiarids are generally referred to being parthenogenetic, Nuttall (1990) reported this condition for *Hemisinus*. However, similar proportions of males and females were found in this study, indicating that sexual reproduction is likely the reproductive mode in Jamaican *Hemisinus*.

According to Strong et al. (in press), both Bayesian and parsimony analyses support Paludomidae and Thiaridae as sister taxa based on molecular and combined molecular/morphological data but not on morphological data alone, which could be product of the lack of information for *Hemisinus*. Consequently, information on *Hemisinus lineolatus* morphology is compared to that of other members of the Thiaridae and Paludomidae (Table 4). Some of the more significant findings of this study relate to new insights into reproductive biology. The male pallial gonoduct in *H. lineolatus* is almost completely open, as in other Thiaridae (Starmühlner, 1969; Muley, 1977). In paludomids, however, the male gonoduct is mostly closed and forms a tubular, anterodorsal spermatophore organ, which typically opens to the anterior third to half of the gonoductal groove. A similar structure, also dorsal but positioned more posteriorly along the axis of the gonoductal groove, is present in *H. lineolatus* (Figs. 50–52). Based on a similar basophilic epithelium to the organ of paludomids, and the presence of sperm (Figs. 57, 58), it is inferred to be a spermatophore-forming organ. However, no spermatophores were found in *Hemisinus*. If homologous in the two families, the structure has been lost by most other members of the Thiaridae, and has become more prominent (i.e., visible externally) and shifted anteriorly in the Paludomidae.

Most thiarids are characterized by an unusual pallial oviduct that is a bilaterally symmetrical, non-glandular tube, with a large, medially placed bursa (Starmühlner, 1969; Schutt & Glaubrecht, 1999; Strong et al., in press). The

TABLE 4. Summary of morphological differences between *Hemisinus lineolatus* compared to other limnic gastropods in the Thiaridae (*Thiara*) and Paludomidae (*Vinundu, Lavigeria, Tiphobia*). Data from Starmühlner, 1969; Schütt & Glaubrecht, 1999; Strong & Glaubrecht, 2002, 2003, 2007, 2010; Michel, 2004; Ben-Ami & Hodgson, 2005. Abbreviations: ct, ctenidium.

	Thiaridae		Paludomidae		
	Hemisinus	*Thiara*	*Vinundu*	*Lavigeria*	*Tiphobia*
Shell					
Juvenile shell	Smooth	Smooth	Granulose	Wrinkled	Wrinkled
External Anatomy					
Operculum	Paucispiral	Paucispiral	Paucispiral	Paucispiral	Concentric, Paucispiral nucleus
Operculum nucleus	Eccentric	Eccentric	Subcentral to eccentric	Subcentral	Subcentral
Mantle edge	Papillate	Papillate	Lobate	Lobate	Smooth
Osphradium length	1/3 ct	~1/2 ct	3/4 ct	~1/2 ct	~1/3 ct
Alimentary System					
Rachidian	Pentagonal/ Broad	Short/Broad	Rectangular	Rectangular	Rectangular
Rachidian basal denticles	Present	Present	Absent	Absent	Absent
Rachidian denticles	2–3/1/2–3	3–6/1/3–5	2/1/2	1/1/1	8–15/1/8–15
Lateral denticles	2–3/1/2–3	2–6/1/2–5	2/1/2	1/1/1	3–6/1/5–16
Marginal denticles	4–5	6–8	5–10	1–5	5–8
Accessory pad	Large, Textured	Large, Textured	Small	Small	Small
Caecal folds	2	2	Absent	1	1
Caecum	Shallow	Deep and spiral	Shallow?	Shallow	Shallow
Style sac typhlosoles	Fused	Fused	?	Partially fused	Partially fused
Reproductive System					
Renal oviduct	Branched	Branched	Unbranched	Unbranched	Unbranched
Pallial oviduct	Glandular	Non–glandular	Glandular	Glandular	Glandular
Spermatophore bursa placement	Lateral lamina	Medial	Medial lamina	Medial lamina	Medial lamina
Spermatophore organ	Mid–dorsal	Absent	?	Anterior	Anterior
Nervous System					
Statocyst	Statoconia	Statolith	?	Statoconia	Statoconia
Right dialyneury/ zygoneury	Dialyneury	Dialyneury	Zygoneury	Zygoneury	Dialyneury

renal oviduct is bifurcate, sending one branch to enter above the bursa, and another to open far anteriorly above the vagina. *Hemisinus* differs from this pattern in possessing a pallial oviduct that is glandular and distinctly asymmetrical, with an albumen gland elaborated posteriorly, and with a large bursa in the lateral lamina. However, the typical thiarid branching pattern of the renal oviduct is retained. The conditions present in *Hemisinus* indicate that the unusual "thiarid" bursa is derived from a sperm storage pouch located within the lateral lamina. The homology to sperm storage pouches in this location in other taxa remains unknown; no sperm storage pouches in the lateral lamina are known in paludomids.

In thiarids, brooding is a common life history trait and is known in all species of the family except *Pachymelania* (Glaubrecht, 1996). Here, the eggs complete their development within a cephalic brood pouch and the young hatch as swimming veligers or crawling juveniles (Muley, 1977). The brood pouch overlies the esophagus in the dorsal cephalic hemocoel, and extends back from a brood pore on the neck. The brood pouch of *H. lineolatus* has the same arrangement but differs from some members of the family (i.e., *Tarebia granifera*, *Thiara scabra*, *T. amarula* and *M. tuberculata*) in its internal structure and in the embryonic developmental stages that are present. Typically, the brood pouch has separate individual compartments formed by columnar epithelium that extends into the brood pouch cavity (Strong & Glaubrecht, 2007; Table 2). Such compartments may be occupied by several early embryonic stages or thousands of developing larvae. In *H. lineolatus*, despite that these kinds of cells are also observed, they do not form compartments and there is no evidence of any early stages inside the pouch.

In thiarids, it has been assumed that energy for embryonic development in the brood pouch may be derived from storage products in the eggs, from the mother or from adelphophagy (Hodgson et al., 2002). Lecithotrophic nourishment of the embryos in freshwater thiarids is known for *Fijidoma*, *Melanoides, Tarebia* and the South American *Aylacostoma* (Strong & Glaubrecht, 2007). Embryonic nourishment in *H. lineolatus* is still unknown.

Affinities of *Hemisinus lineolatus*

Morrison (1951), based on operculum and external head-foot features, included *Hemisinus* within the Thiaridae, a view that has not been changed since. Comparison of *Hemisinus lineolatus* with thiarids and paludomids (Table 4), shows that *Hemisinus* exhibits many typical thiarid characteristics, such as the structure of the operculum, mantle edge, rachidian (shape, basal denticles), midgut (large, textured accessory pad; two short, prominent caecal folds; fused typhlosoles), renal oviduct (branching), and cephalic brood pouch. It also shares some anatomical similarities with paludomids (statoconia, spermatophore organ), especially basal paludomids (mantle papillae, right dialyneury) (Strong, unpubl. data). Some of the anatomical differences between *Hemisinus* and other thiarids (e.g., glandular pallial oviduct with bursa in lateral lamina) could indicate that it does not group within the Thiaridae. However, it is more parsimonious to conclude that the features shared with paludomids, in combination with its unique anatomical attributes, are consistent with a basal position within the Thiaridae, a position thus far supported in phylogenetic analyses of the superfamily, albeit with limited taxonomic sampling and incomplete anatomical information (Lydeard et al., 2002; Strong et al., in press).

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NEACTAEONINA ARGENTINA, NEW SPECIES, AND FAMILY PLACEMENT OF THE GENUS *NEACTAEONINA* THIELE, 1912 (MOLLUSCA: GASTROPODA)

Diego G. Zelaya[1, 2*], Laura Schejter[3] & Cristián Ituarte[4]

ABSTRACT

Neactaeonina argentina, a new species from the outer Argentine shelf off Buenos Aires province, is described. The species is characterized by the elongate, low-spired shell, with shouldered, nearly straight whorls, deep suture, and sculpture composed of numerous, broad, flat spiral cords. The radula is similar to that previously described for the type species, *Neactaeonina cingulata* Thiele, 1912, except in having a greater number of teeth. The present study provides the first information on the anatomy and histology of the genital system, and details of the radula anatomy of *Neactaeonina*, both unknown to date. The study of anatomical characters demonstrates that *Neactaeonina* has clear similarities with other Acteonidae, with a combination of features present in *Acteon* (such as the presence of a strongly calcified shell, with well-developed operculum; mantle cavity opening anteriorly; nervous system with only the cerebropleural ganglia fused; a single allosperm sac associated with the female system; radula without cardinal tooth; and oral cuticle with rod-like processes); and some characters present in acteonid genera other than *Acteon* (e.g., the number and morphology of teeth, and processes of the oral cuticle). However, *Neactaeonina* shows some characters thus far not reported for any other genus currently placed into the Acteonidae: salivary glands with laminar structure, male genital system with an open portion, a single allosperm sac opening distally, and a flat and triangular copulatory organ.

Keywords: Gastropoda, Acteonidae, *Neactaeonina*, Southern Ocean.

INTRODUCTION

Neactaeonina was proposed by Thiele (1912) to include three species from sub-Antarctic and Antarctic waters: *Actaeonina cingulata* Strebel, 1908 (type species) from South Georgia; *Actaeon* (sic) *edentulus* Watson, 1889 from Kerguelen Islands, and *Neactaeonina fragilis* Thiele, 1912, from the Davis Sea of eastern Antarctica. Subsequently, Dell (1956) described a fourth species, *Neactaeonina inexpectata*, from Chatham Rise, off New Zealand.

The descriptions of sub-Antarctic and Antarctic *Neactaeonina* species were based on subtle morphological shell characters observed in few specimens, if not on a single specimen. Differential characters were never clearly described, resulting in a confusing taxonomy and mistaken identifications. In this regard, for instance, Powell (1951) reported specimens from South Georgia and South Shetlands Islands as *Neactaeonina edentula*, but latter (Powell, 1960) considered that these records correspond either "to [*N.*] *cingulata* or to a new species".

The familial assignment of the genus has never been properly discussed. Odhner (1926), based on the radula morphology described by Thiele (1912), placed *Neactaeonina* in the Acteonidae. New information on anatomy and radular morphology of a new species of *Neactaeonina* from the Argentine continental shelf described here, allowed a revision of the systematic position of the genus.

MATERIALS AND METHODS

The specimens described here were collected by the BIP "Capitán Cánepa" (Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP) during routine cruises for stock assess-

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[1] División Zoología Invertebrados, Museo de La Plata, Paseo del Bosque s/n, 1900, La Plata, Argentina
[2] Departamento Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, UBA, Argentina
[3] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Paseo Victoria Ocampo 1, BH7602 Mar del Plata, Argentina
[4] Museo Argentino de Ciencias Naturales, Ángel Gallardo 470, C1405DJR Ciudad de Buenos Aires, Argentina
*Corresponding author: dzelaya@fcnym.unlp.edu.ar

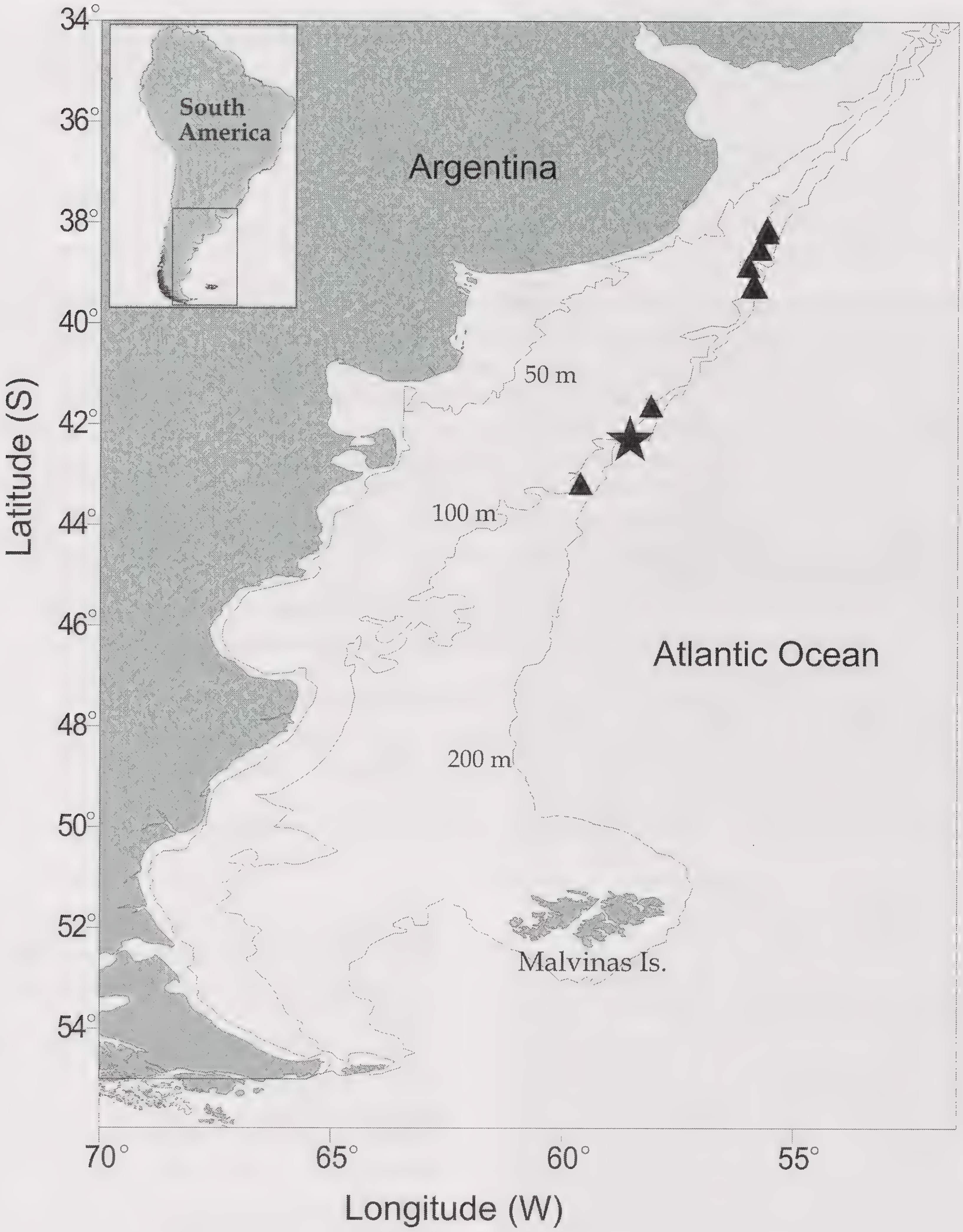
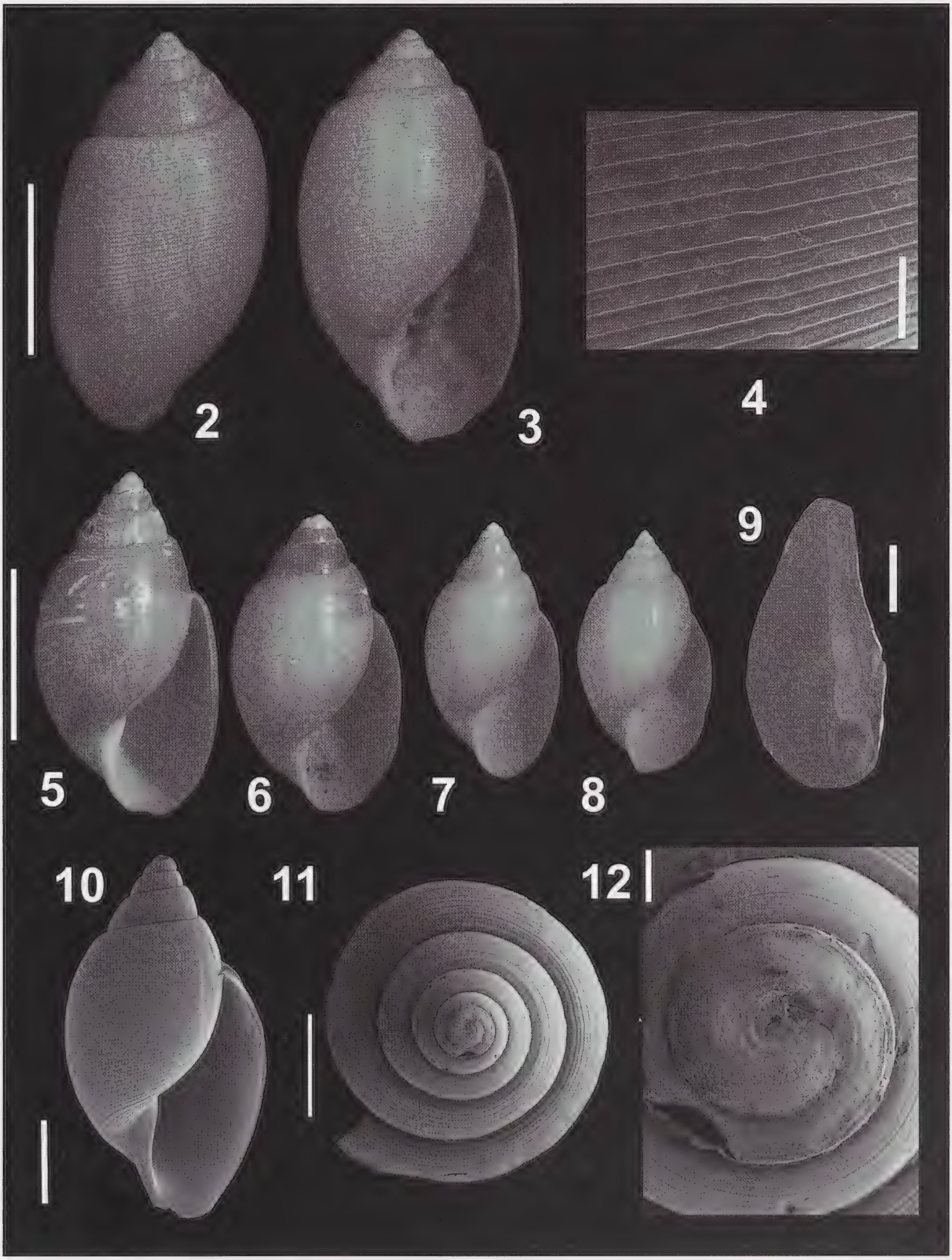


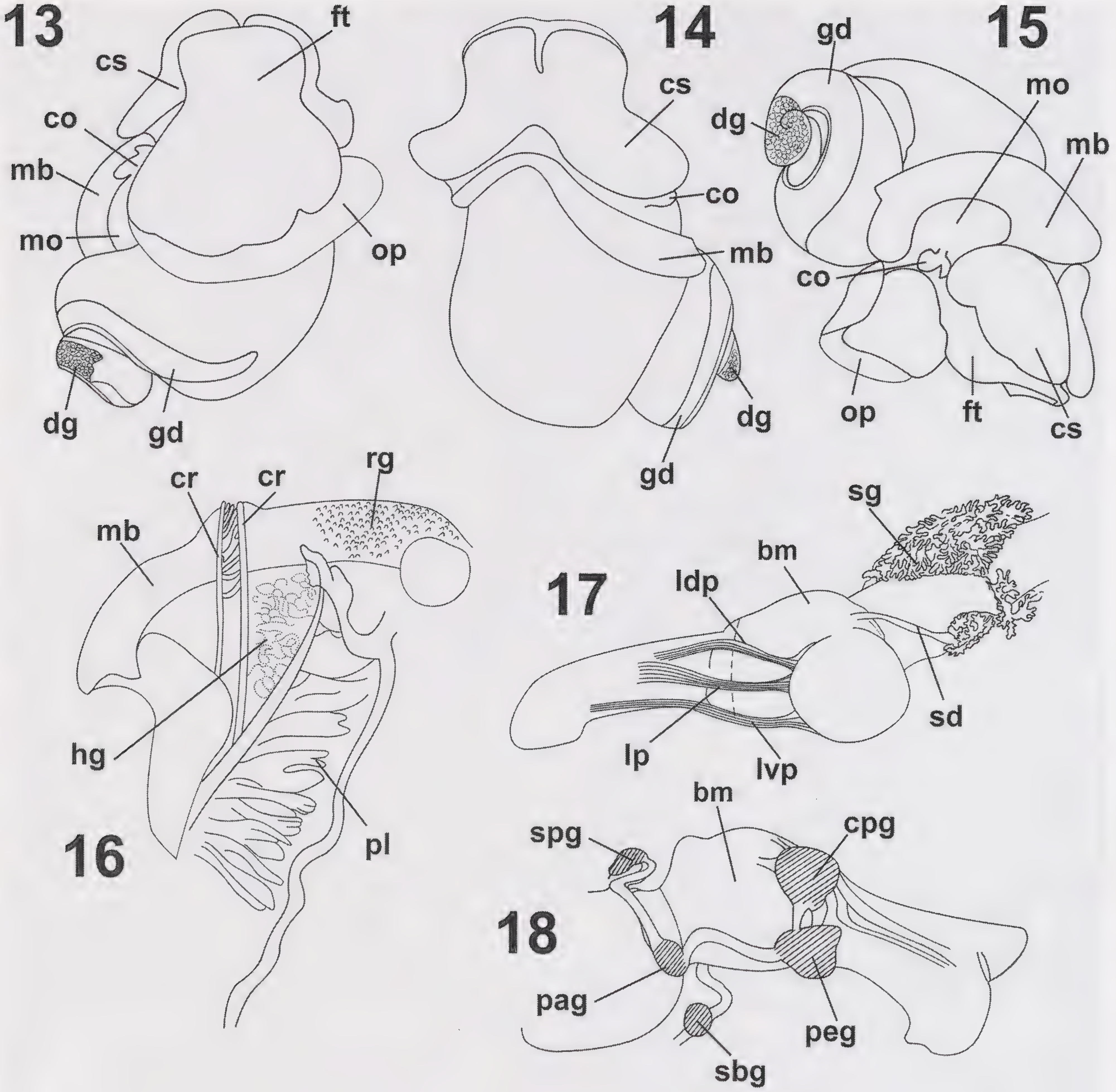
FIG. 1. Location map. Type locality (★); other material examined (▲)

ment of the Patagonian scallop *Zygochlamys patagonica* (King, 1832). Benthic samples were collected using a non-selective dredge (2.5 m mouth aperture). Gastropods were sorted, fixed in a 5% formalin solution, and preserved in 75% alcohol. Voucher specimens were deposited in the malacological collection at Museo de La Plata (MLP), La Plata and Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN), Buenos Aires. For comparative purposes, the types or photographs of the type specimens of other species of *Neactaeonina* were examined: Museum of New Zealand Te Papa Tongarewa (NMNZ), Wellington, New Zealand [*N. inexpectata*]; The Natural History Museum (BMNH), London, United Kingdom [*N. edentula*]; Swedish Museum of Natural History (SMNH), Stockholm, Sweden [*N. cingulata*]; and Zoologisches Museum (ZMB), Berlin, Germany [*N. fragilis*].

Gross anatomy was studied by dissection under a stereoscopic microscope; the radula was dissected, cleaned by rising in a commer-



FIGS. 2–12. ***Neactaeonina argentina,*** new species. FIGS. 2, 3: Holotype (MLP 13264). FIG. 2: Dorsal view; FIG. 3: Apertural view. FIGS. 4–8: Paratypes. FIG. 4: Detail of shell sculpture; FIGS. 5–8: Ontogenetic series; FIG. 9: Operculum. FIGS. 10–12: Specimen from 38° 09.88' S, 55° 35.42' W (MACN-In 38141). FIG. 10: Apertural view (SEM); FIG. 11: Apical view; FIG. 12: Detail of protoconch. Scale bars FIGS. 2, 3, 5–8 = 10 mm; FIGS. 4, 12 = 200 µm; FIG. 9 = 1 mm; FIGS. 10, 11 = 2 mm.



FIGS. 13–18. ***Neactaeonina argentina,*** new species: gross anatomy. FIG. 13: Ventral view; FIG. 14: Dorsal view; FIG. 15: Lateral view; FIG. 16: Detail of the roof of the pallial cavity; FIG. 17: Anterior part of digestive system; FIG. 18: Nervous system. bm: buccal mass; co: copulatory organ; cpg: cerebropleural ganglion; cr: ciliated ridge; cs: cephalic shield; dg: digestive gland; ft: foot; gd: gonad; hg: hypobranchial gland; ldp: laterodorsal protractor muscle; lp: lateral protractor muscle; lvp: lateroventral protractor muscle; mb: mantle border; mo: mantle opening; op: operculum; pag: pallial ganglion; peg: pedal ganglion; pl: plicatidium; rg: repugnatorial glands (?); sbg: subesophageal ganglion; sd: salivary duct; sg: salivary gland; spg: suprapharyngeal ganglion.

cial sodium hypochlorite solution, washed in distilled water, and prepared dried for Scanning Electron Microscopy (SEM). Histological serial sections of three specimens were obtained after embedding tissues in Paraplast® (7 µm thick) and Historesin® (3.5 µm thick), and stained with eosin-hematoxylin.

SYSTEMATICS

***Neactaeonina argentina*, new species**

Figs. 1–50

Type Locality: 42°14.12'S, 58°32.97'W, off Buenos Aires province, 95 m depth.

Type Material: Holotype (23.5 mm length) (MLP 13264) and 4 paratypes from 38°33.82'–38°34.96'S, 55°44.63'–55°46.71'W, 84–91 m (2 paratypes, MLP 13265; 2 paratypes MACN-In 38140).

Other Material Examined: Off Buenos Aires province at: 1 specimen, 38°09.88'S, 55°35.42'W, 94 m (MACN-In 38141); 5 specimens, 38°55.30'S, 55°59.86'W, 84 m (MLP 13266); 1 specimen, 39°19.86'S, 55°54.62'W, 105 m (MLP 13267); 1 specimen, 43°14.18'S, 59°37.27'W, 100 m (MLP 13268); 1 shell, 41°41.95'S, 58°05.70'W, 96 m (MLP 13269); 1 specimen, 38°15.13'S, 55°35.55'W, 97 m (MACN-In 38142).

Etymology

The species name refers to Argentina, the country from whose continental shelf the species was collected.

Diagnosis

Shell solid, spire low, somewhat acute, weakly shouldered and nearly straight-sided whorls, with deep suture. Sculpture of numerous, broad, flat spiral cords. Columellar callus wide. Radular formula: 27–0–27.

Description

Shell of medium size (maximum observed length = 23.5 mm), strongly calcified, globose, elongated, with apex exposed (Figs. 2, 3, 5–8). Protoconch of 1.5 whorls, about 960 μm diameter (Fig. 12). Teleoconch of up to 4¾ whorls. Spire acute, high, with almost straight and shouldered whorls; suture deep (Figs. 2, 3, 5–8, 10–12). Last whorl enlarged, ovate, inflated, particularly in small specimens (Figs. 2, 3, 5–8, 10, 11). Aperture elongate, about 65–70% of shell high, wider anteriorly. Outer lip thin, evenly curved. Columella slightly arcuate, nearly straight at the base, smooth. Columellar callus narrow, white, stronger anteriorly. Shell sculptured with broad, flat, regularly spaced spiral cords (Fig. 4), varying from 11–12 in the first whorl of teleoconch, 14–17 in the second whorl, 18–23 in the third whorl, and 72–76 in the last adult whorl. Each cord bearing microscopic striations. Spiral cords sinuous. Interspaces narrower than cords, evidencing growth lines, particularly anteriorly (Fig. 4). Shell surface glossy, straw yellowish. Operculum elongate, paucispiral, with basal nucleus, widely curved at external margin, angulated at inner margin, yellowish (Fig. 9).

Anatomy

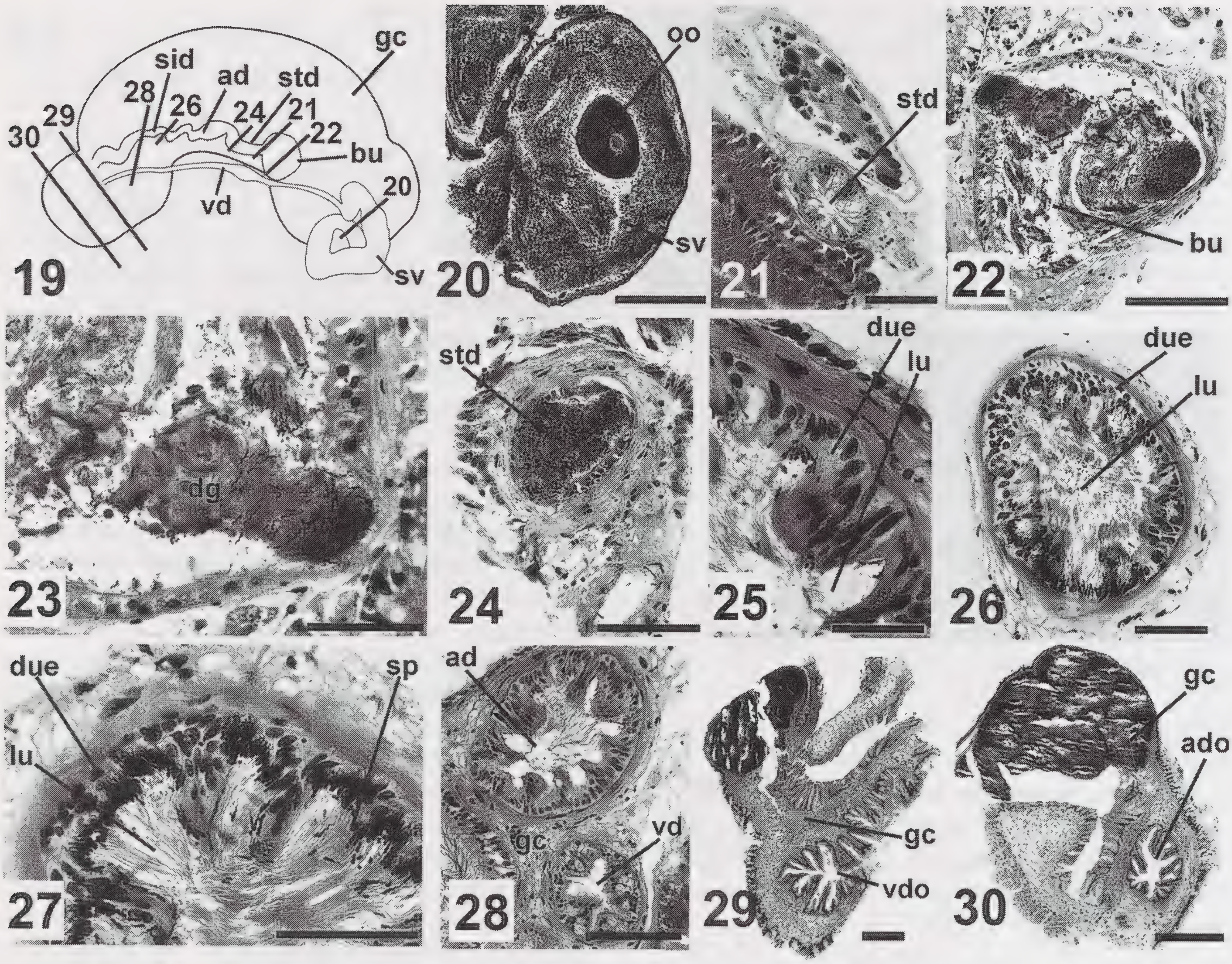
Animal able to completely retract into the shell.

Head-Foot: Eyes absent. Foot relatively small, ovate; anterior end with projecting triangular lateral horns; posterior end widely rounded (Fig. 13). Parapodia absent. Anterior part of cephalic shield rounded; narrow, deep medial sinus, present. Posterior part projecting in two widely separated lobes reaching base of mantle cavity (Figs. 14, 15).

Mantle Cavity: Opening anteriorly, slightly displaced to right (Figs. 13, 15). Mantle border bent dorsally, left side with small papillae on ventral surface (repugnatorial glands?) (Fig. 16). Temporary "siphon" formed by small, triangular, smooth expansion of right mantle border. Plicatidium-like gill comprising few convolute leaflets (Fig. 16), attached at both edges of roof mantle cavity; right anterior part free for about half of its length; left anterior part free for about a sixth of its length. Ciliated ridge ("raphe"), running along roof of mantle cavity, at right of gill (Fig. 16). Second raphe on floor of mantle cavity, present. Both raphes extend posteriorly beyond mantle cavity, running to end of spire, inside pallial caecum. Hypobranchial gland difused on roof of pallial cavity, between dorsal raphe and gill (Fig. 16). Kidney relatively large, running along left side of gill. Heart one half kidney size; auricle anterior to ventricle.

Reproductive System: Gonad with hermaphroditic acini; oocytes developing at blind end of acini. Short hermaphroditic duct connecting with seminal vesicle (also called "ampulla"), serving for autosperm storage prior to ejaculation and for passage of ova toward female glandular complex, present (Fig. 19). Seminal vesicle large, elongate, sigmoid sac, lined by simple epithelium of cubic or low columnar cells (about 12 μm height) (Figs. 19, 20). Interior wall of vesicle facing columella ciliated.

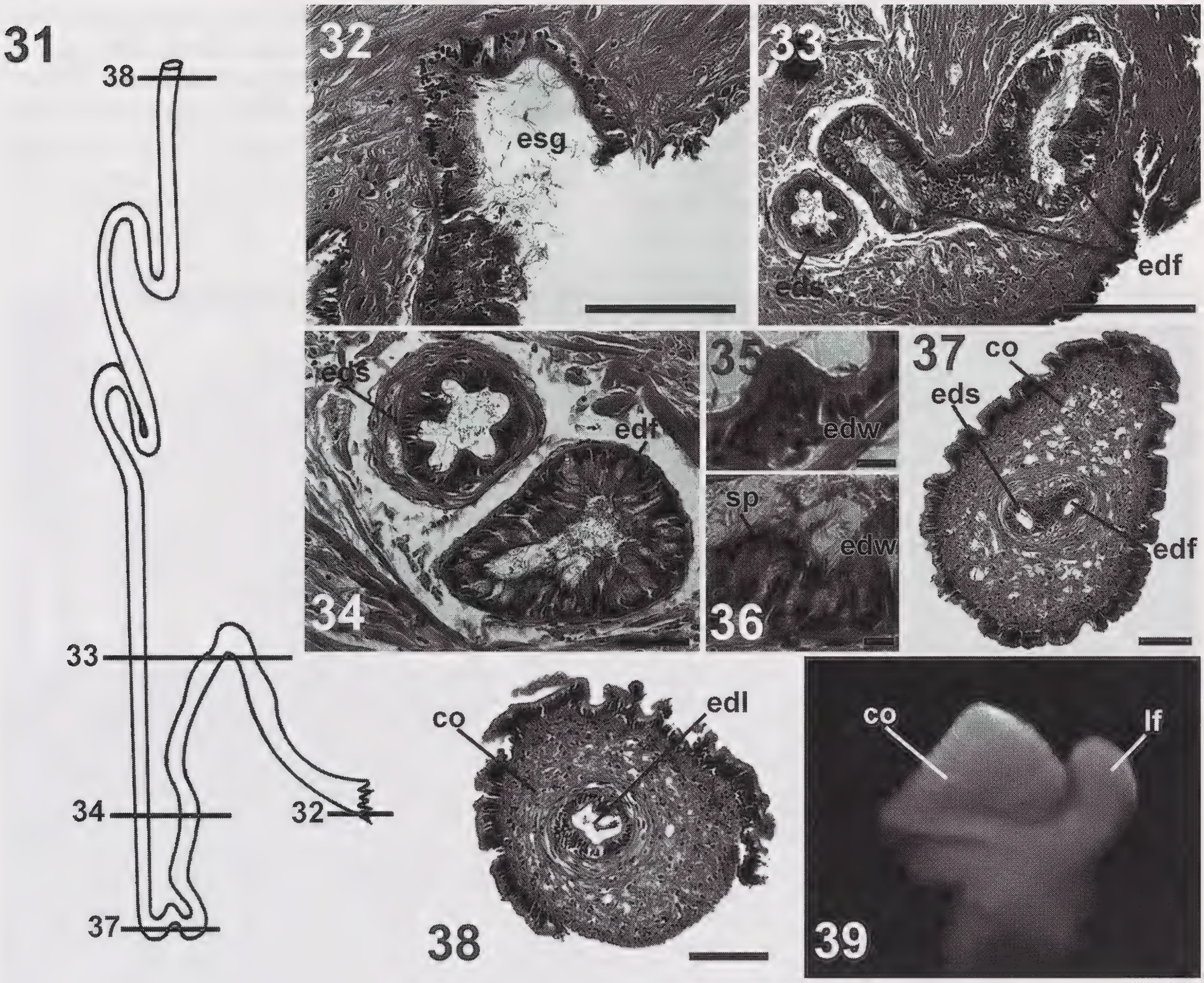
Female System: Glandular complex large, elongate, composed of two externally discernible glandular areas (Figs. 19, 29, 30). Single (allosperm) duct opening distally and running parallel to left side of female gland complex (Fig. 19). Allosperm duct with uniform diameter throughout its length; first three-quarters sinuous, becoming straight before opening into small globular sac ("gametolytic gland") (Fig. 21). Histologically, bulb wall composed



FIGS. 19–30. ***Neactaeonina argentina***, new species: proximal portion of male and female reproductive systems. FIG. 19: Diagram (numbers indicate the histological sections showed in FIGS. 20–30); FIG. 20: Seminal vesicle; FIG. 21: Allosperm duct just before opening into the bulb; FIG. 22: Bulb; FIG. 23: Detail of degenerating gametes within bulb; FIG. 24: Straight portion allosperm duct; FIG. 25: Detail of FIG. 24 showing the duct epithelium; FIG. 26: Sinuous portion of allosperm duct; FIG. 27: Detail of FIG. 26 showing sperm attached to the duct epithelium; FIG. 28: Allosperm duct and vas deferens immersed in the terminal portion of the glandular complex; FIG. 29: Opening of the vas deferens; FIG. 30: Distal opening of allosperm duct. Scale bars: FIGS. 20, 29 = 200 μm; FIGS. 21, 22, 28 = 100 μm; FIGS. 23–27 = 50 μm; FIG. 30 = 30 μm. ad: allosperm duct; ado: allosperm duct opening; bu: bulb; dg: degenerating gametes; due: duct epithelium; gc: glandular complex; lu: lumen; oo: oocyte traversing the seminal vesicle; sid: sinuous portion of the duct; sp: sperm; std: straight portion of the duct; sv: seminal vesicle; vd: vas deferent; due: duct epithelium; vdo: vas deferent opening.

of low columnar cells (about 15 μm height), with vacuolated cytoplasm suggesting some involvement in reabsorption (Figs. 22, 23). Distinct muscular layer absent; lumen usually containing unordered sperm and debris of degenerating gametes which appears as eosinophilic spherules (Figs. 22, 23). Epithelium of allosperm duct formed by single layer of cubic cells, supported by thin connective tissue, ensheathed by somewhat thick layer of circular muscle (Figs. 25–28). Straight portion of duct containing unoriented sperm, whereas in sinuous portion, sperm oriented with heads perpendicular to wall of duct (Figs. 24–27). At distal end, duct of allosperm bulb joining lumen of female duct, to open shortly thereafter close to female genital opening (Fig. 30).

Male System: Vas deferens, arising from seminal vesicle, running anteriorly, parallel to female gland and ventral to allosperm duct (Fig. 19). Epithelium of vas deferens formed by high columnar cells forming several ridges into lumen; duct ensheathed by somewhat thick muscular layer. Vas deferens entering distally into glandular female complex; just before entering duct



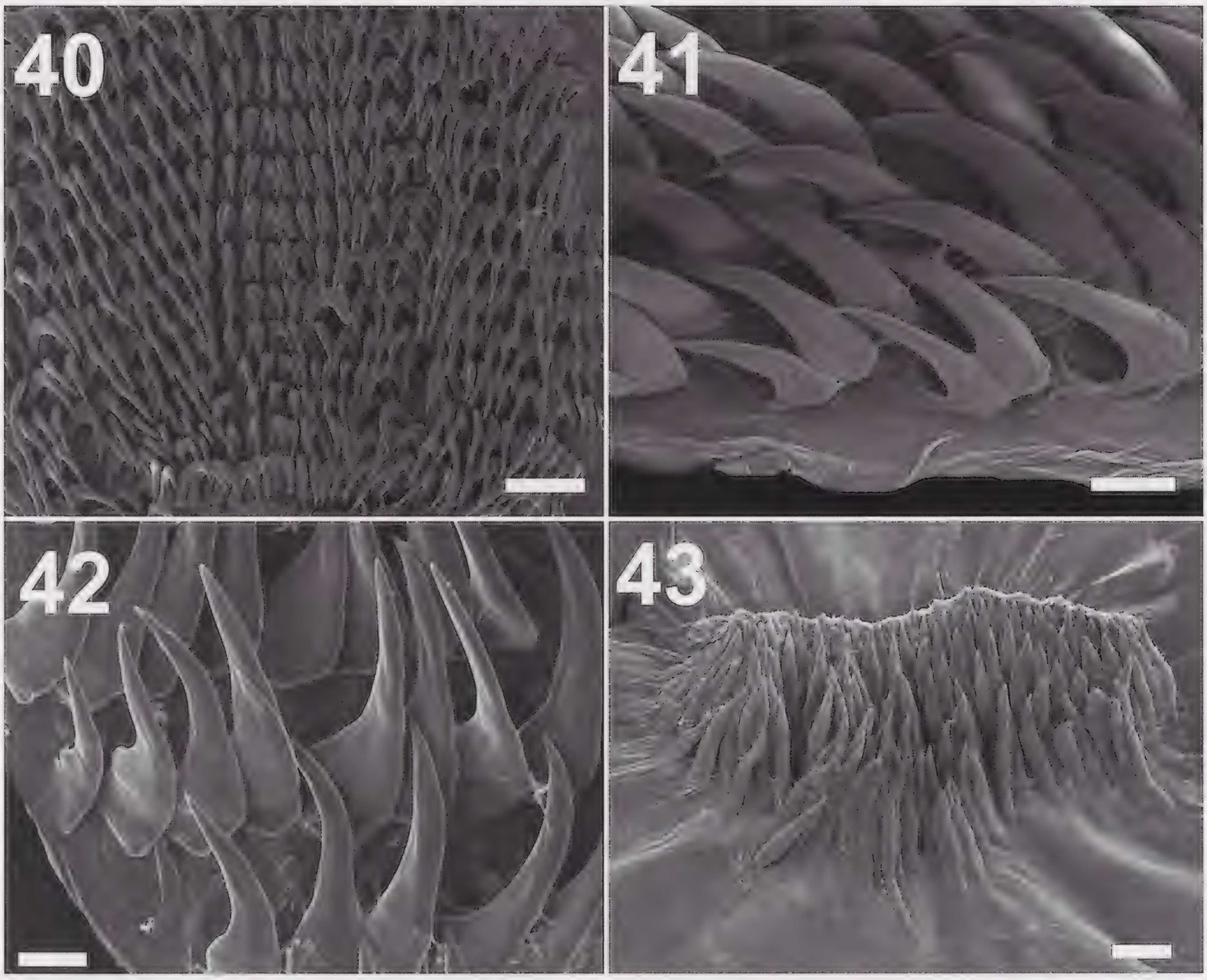
FIGS. 31–39. ***Neactaeonina argentina,*** new species: distal portion of male reproductive system. FIG. 31: Schematic drawing (not in scale) of the ejaculatory duct (lines and numbers indicate the histological sections showed in FIGS. 32–34, 37, 38); FIG. 32: External seminal groove; FIGS. 33, 34: Transverse sections of first and second half of ejaculatory duct; FIG. 35: Detail of the second half; FIGS. 36: Detail of the first half showing sperm attached to the duct wall; FIG. 37: Junction of first and second half of ejaculatory duct; FIG. 38: Distal part of ejaculatory duct, close to the tip of the copulatory organ; FIG. 39: Copulatory organ. Scale bars: FIGS. 32, 37, 38 = 100 µm; FIG. 33 = 200 µm; FIG. 34 = 50 µm; FIGS. 35, 36 = 10 µm. co: copulatory organ; edf: first part of ejaculatory duct; edl: ejaculatory duct lumen; eds: second part of ejaculatory duct; edw: ejaculatory duct wall; esg: external seminal groove; lf: lateral flap; sp: sperm.

epithelium lined by large cells with vesiculous cytoplasm (Fig. 28). Shortly after junction of vas deferens and lumen of female glandular complex, vas deferens opening into exterior slightly anterior to female genital opening (Fig. 29). From this point to the base of copulatory organ, male way open, forming ciliated groove (Fig. 32). At base of copulatory organ, closed U-shaped ejaculatory duct opening, after several convolutions, at tip of the copulatory organ (Fig. 31). Just after entering base of copulatory organ, ejaculatory duct enlarges for short length, to continue with U-shaped branch. First half of U-shaped duct lined by high columnar glandular epithelium (Figs. 33, 34, 36), with sperm embedded in epithelium (Fig. 36). Duct of second half of U and distal convoluted portion lined by columnar, non-glandular epithelium of variable height, forming five or six ridges toward lumen (Figs. 33, 34, 36, 37). Ejaculatory duct ciliated along its entire length, surrounded by muscular sheath (Fig. 38). Prostatic tissue associated with ejaculatory duct not observed. Copulatory organ triangular, non-retractile, unarmed (Fig. 39). A ventral fold (like a prepuce) at base of copulatory organ expanding into lateral, foliated, anteriorly directed flap (Fig. 39).

Digestive System: Oral tube short. Buccal mass bulbous, strongly muscular, with three pairs of lateral protractor muscles (laterodorsal, lateral, and lateroventral) (Fig. 17). Pair of large salivary glands, ventrolaterally embracing esophagus (Fig. 17). Salivary glands open at posterodorsal end of buccal mass, above radular ribon. Left salivary duct relatively long; right salivary duct short. Salivary glands flat, highly branched. Posterior portion of esophagus enlarged, forming crop. Stomach saculate, surrounded by female glandular complex. Digestive gland externally visible only at last whorl of visceral mass (Figs. 13–15). Intestine opening at anterior part of stomach, close to esophagus opening. Intestine relatively short, crossing floor of mantle cavity, and running at right side of female glandular complex to open at base of temporal "siphon".

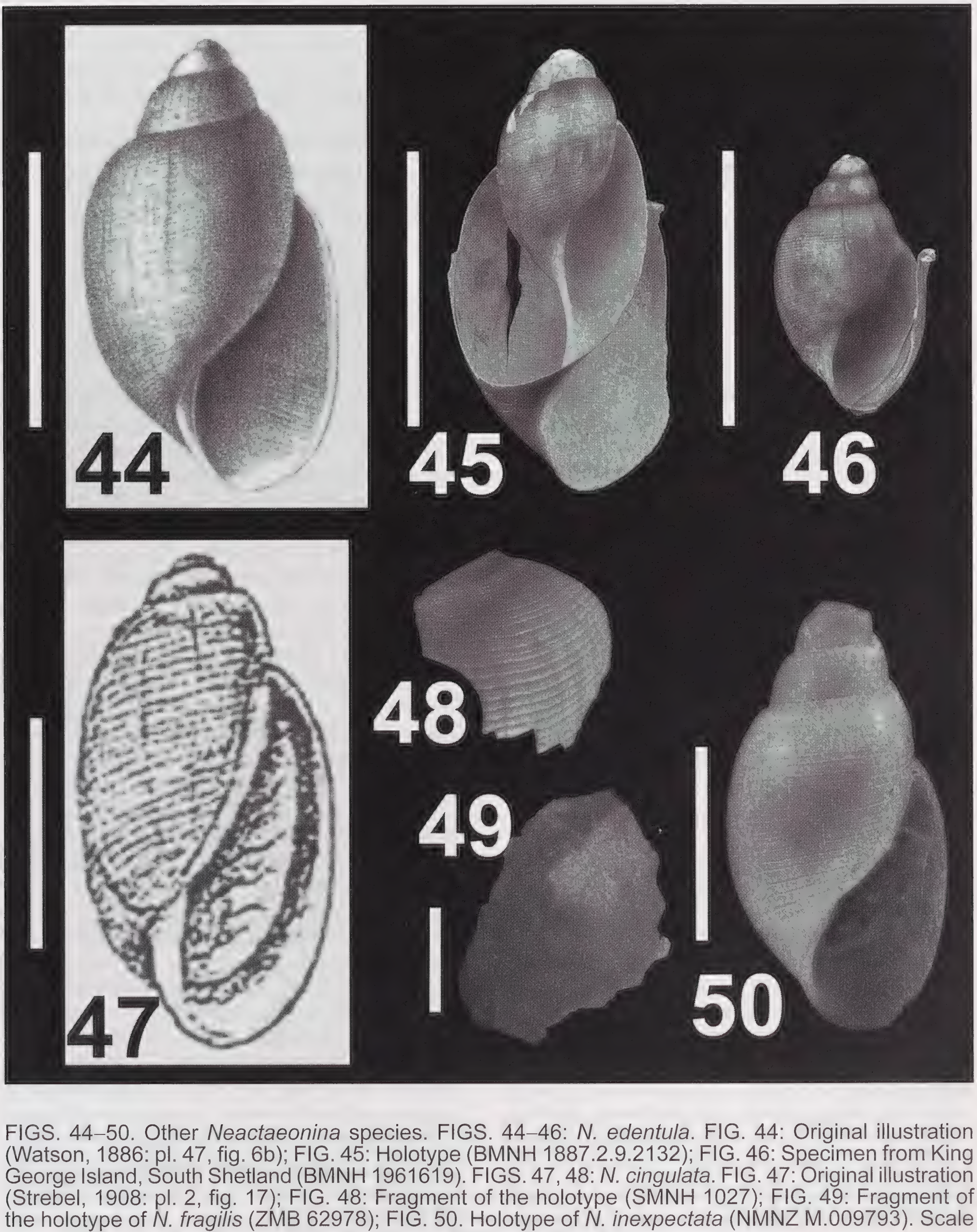
Radula: Composed of approximately 30 rows of 27 teeth each. Rachidian tooth absent. Lateral teeth long, prominently hooked, sharply pointed, smooth, similar in size and shape, except for two outermost rows, where teeth are smaller and less solid, each bearing one or two tubercles close their bases (Figs. 40–42); base of teeth flat, discoidal, solid. Oral cuticle with numerous rows of somewhat irregular, sharply pointed rod-like processes (Fig. 43).

Nervous System: Streptoneurous. Nerve ring prepharyngeal, composed of four large ganglia: one dorsal pair, originating from fusion of cerebral and pleural ganglia, and ventral pair, the pedal ganglia. Right and left cerebropleural ganglia connected by long, thick commissure; pedal ganglia connected by very short commissure; connectives between cerebropleural



FIGS. 40–43. ***Neactaeonina argentina***, new species. FIGS. 40–42: Radula. FIG. 40: General morphology; FIGS. 41, 42: Detail of lateral teeth. FIG. 43: Oral cuticle. Scale bars: FIG. 40 = 100 µm; FIGS. 41–43 = 20 µm.

and pedal ganglia double, short (Fig. 18). Pallial ganglia small, located at sides of buccal mass, clearly separated from cerebropleural and esophageal ganglia by long connectives. Right esophageal ganglion smaller than left, which is represented by only slight enlargement of connective; left connective running below esophagus, joining well-developed subesophageal ganglion (Fig. 18); right connective running over esophagus, where it joins supraesophageal ganglion. From subesophageal and supraesophageal ganglia, two connectives run posteriorly, passing below alimentary canal, to join visceral ganglion.



FIGS. 44–50. Other *Neactaeonina* species. FIGS. 44–46: *N. edentula*. FIG. 44: Original illustration (Watson, 1886: pl. 47, fig. 6b); FIG. 45: Holotype (BMNH 1887.2.9.2132); FIG. 46: Specimen from King George Island, South Shetland (BMNH 1961619). FIGS. 47, 48: *N. cingulata*. FIG. 47: Original illustration (Strebel, 1908: pl. 2, fig. 17); FIG. 48: Fragment of the holotype (SMNH 1027); FIG. 49: Fragment of the holotype of *N. fragilis* (ZMB 62978); FIG. 50. Holotype of *N. inexpectata* (NMNZ M.009793). Scale bars: FIGS. 44–46 = 30 mm; FIG. 47 = 5 mm; FIGS. 49, 50 = 2 mm.

Known Distribution

38°09.88'–43°14.18'S, 55°35.42'–59°37.27'W, off Buenos Aires Province; 84–126 m (Fig. 1).

Habitat

Neactaeonina argentina, n. sp., was found in soft-bottoms, being part of the benthic assemblage of the Patagonian scallop *Zygochlamys patagonica*.

DISCUSSION

Comparison of ***Neactaeonina argentina***, n. sp., with other *Neactaeonina* Species

Neactaeonina argentina, n. sp., is most similar to *N. edentula* (Figs. 44–46), from which it differs in having a more acute spire with shouldered whorls and deeply channeled suture. According to Watson (1886) sutures in *N. edentula* are "axially impressed rather than channeled".

Neactaeonina argentina, n. sp., differs from *N. cingulata* (Figs. 47, 48) in having a lower spire, and a different number of lateral teeth. Thiele (1912) described the radula of *N. cingulata* as having one central tooth and 7 to 8 laterals at each side – interpreted as 8.0.8 by Hoffmann (1932–1940) – whereas ***N. argentina***, n. sp., has 27 laterals in each row. The morphology of the lateral teeth, however, shows no differences in ***N. argentina***, n. sp., and *N. cingulata*.

Compared with *Neactaeonina inexpectata* (Fig. 50), ***N. argentina***, n. sp. has a more solid shell, sculptured with a greater number of spiral cords and a wider columellar callus. Furthermore, in *N. inexpectata* the spire whorls are rounded, whereas in ***N. argentina***, n. sp., whorls are nearly straight.

The affinities between ***Neactaeonina argentina***, n. sp., and *N. fragilis* are difficult to establish. The original description of *N. fragilis* is poor, lacking in detail and illustration. The type material consists of fragments of a single shell (Fig. 49), which show a shell thinner than in ***N. argentina***, n. sp., with more delicate sculpture. After the original description, the only subsequent references to this species are those by Hedley (1916) and Hain (1990), who tentatively assigned to *N. fragilis* specimens from Adelia Land and Weddell Sea, respectively. Hedley (1916) did not describe or figure the studied specimens, and Hain (1990: plate 8, fig. 9) only figured the general shell outline of a single specimen which differs from ***N. argentina***, n. sp., in having a depressed spire. Dell (1990) pointed out that "[*Neactaeonina*] *fragilis* may ultimately prove to be indistinguishable from [*N.*] *edentula*".

The overall similarities in shell morphology, radula, and oral cuticle of ***Neactaeonina argentina***, n. sp., and *N. cingulata*, the type species of the genus, clearly support the generic status of the new species. Information on the soft part anatomy of the other *Neactaeonina* species is unknown.

Suprageneric Placement of *Neactaeonina*

Although the text by Thiele (1912) containing the description of *Neactaeonina* is printed immediately following the text corresponding to *Acteon antarcticus*, the familial placement of this genus was not formally proposed until 14 years later, when Odhner (1926) placed it into the Acteonidae. This was followed by all subsequent authors (Thiele, 1931 [as Actaeonidae]; Powell, 1951, 1960; Carcelles, 1953; Dell, 1990; Hain, 1990 [as Acetonidae, by misspelling]); however, the generic placement of *Neactaeonina* was never properly justified. In fact, Odhner (1926) only pointed out some differences between *Neactaeonina* and *Acteon* Montfort, 1810 (the type genus of Acteonidae), not discussing on the similarities that allowed him to unite the two genera in the same family.

The Acteonoidea were traditionally regarded as comprising three families: Acteonidae, Aplustridae, and Bullinidae (Rudman, 1972a; Burn & Thompson, 1998; Bouchet & Rocroi, 2005). Göbbeler & Klussmann-Kolb (2010), performed the first phylogenetic analysis of these families, concluding that only two of these clades appear well-supported: Acteonidae (without *Rictaxis*) and Aplustridae (including *Bullina* and *Rictaxis*). The characters previously described for *Neactaeonina* may be therefore now traced in Göbbeler & Klussmann-Kolb's (2010) phylogeny.

Is *Neactaeonina* Actually an Acteonid?

Neactaeonina has an elongate, strongly calcified shell, with well-developed operculum; a small and unlobed foot; a short oral tube; absence of central radular tooth; mantle cavity opening anteriorly; and nervous system with long conective between the supraesophageal ganglion and the right cerebro-pedal ganglia. It is thus evident that *Neactaeonina* exhibits the characters reported as diagnostic for the Acteonidae.

Comparison of *Neactaeonina* with other Acteonids

The radula of *Neactaeonina* clearly differs from that of *Acteon* both in the number and morphology of teeth. *Acteon* species have numerous, minute, denticulate, and identical teeth – radular formula: 100+.0.100+ (Mikkelsen, 1996); 50–150.0.50–150 (Gosliner, 1996). In contrast, *Neactaeonina* species have a minor number of teeth, with the two outermost rows smaller; the teeth in *Neactaeonina* species are larger and almost smooth (except for one or two tubercles close to the bases of the outermost laterals). The radulae of *Neactaeonina* species closely resemble that of *Crenilabrum exilis* (Jeffreys, 1870), *Japonacteon pusillus* (Forbes, 1843), and *Maxacteon cratericulatus* (Hedley, 1906), respectively described by Bouchet (1975: fig. 1A), Bouchet (1975: fig. 3), and Rudman (1972a: fig 8A): all of them have sharply pointed laterals. However, in *Neactaeonina*, such as in *Japonacteon* and *Maxacteon*, the rachidian tooth is absent, whereas in *C. exilis* (according to Bouchet, 1975), this tooth is present, although reduced at the base. *Japonacteon* has differentiated marginal and lateral teeth, and *Maxacteon* has bicuspid laterals.

The oral cuticle in *Neactaeonina* is composed of rod-like processes resembling lateral teeth, whereas in *Acteon* species these processes are multicusped (Marcus, 1972; Gosliner, 1994, 1996: fig. 2.3B). The oral cuticle of *Neactaeonina* is similar to that described by Bouchet (1975: fig. 1C) for *Crenilabrum exilis*, but unlike those of *Pupa* species (described by Rudman, 1972a), which are similar to those of *Acteon* species.

The opening of the single allosperm sac (composed of a seminal receptacle in tandem with the gametolytic gland) in ***Neactaeonina argentina***, n. sp., is distally with respect to the gonad (i.e., near the genital opening), whereas in *Acteon* species the opening is proximal (i.e., near the gonad), at the level of the seminal vesicle (Johannson, 1954; Fretter & Graham, 1954; Ghiselin, 1966; Gosliner, 1981). According to Gosliner (1994), the presence of only one sperm storage "organ" is a derived condition for the acteonids; the primitive condition for the Actaeonoidea is the presence of two allosperm sacs: the proximal, functioning as seminal receptacle, and the distal serving as gametolytic gland, as for example in *Pupa* and *Maxacteon* species (Rudman, 1972b; Gosliner, 1981).

The male system in ***Neactaeonina argentina***, n. sp., is peculiar, resulting from the combination of a closed spermatic duct running alongside the female glandular complex (the vas deferens) and an external groove, carrying the sperm from the end of the vas deferens to the base of the copulatory organ; from this point, another closed portion (the ejaculatory duct) carry sperm to the tip of the copulatory organ. Interestingly, the first (glandular) portion of the ejaculatory duct seems to be involved in the temporary sperm storage before ejaculation. In *Acteon* species, the male genital duct is closed, and the vas deferens is continuous with the ejaculatory duct (Fretter & Graham, 1954; Johansson, 1954). The latter condition was also reported for *Pupa* and *Maxacteon* species (Rudman, 1972b), and was previously regarded as characteristic of the Acteonoidea (Gosliner, 1994).

Associated with the vas deferens, a glandular portion forming a prostate (the so-called "pallial prostate") was described for some acteonids (Johannson, 1954; Fretter & Graham, 1954; Gosliner, 1994; Mikkelsen, 1996). In ***Neactaeonina argentina***, n. sp., no prostatic tissue associated with any portion of the vas deferens, was observed.

The copulatory organ in *Acteon* species is a straight, tubular structure (Marcus, 1972, 1974), whereas in ***Neactaeonina argentina***, n. sp., the copulatory organ is a triangular and somewhat flattened structure. Fretter & Graham (1954) reported in *Acteon tornatilis* the presence of a shallow basal fold, like a prepuce; a similar structure is also present in ***N. argentina***, n. sp. (this study).

In *Neactaeonina* species the salivary glands are profusely branched, forming flat or laminar structures embracing part of the esophagus. In *Acteon* species, as in *Pupa, Maxacteon*, and *Crenilabrum* species, the salivary glands are tubular, elongate, and running parallel to the esophagus (Rudman, 1972a, b; Bouchet, 1975; Gosliner, 1994).

Neactaeonina argentina, n. sp., lacks eyes, whereas *Acteon* has well-developed eyes.

In shell morphology, species of *Neactaeonina* clearly differ from those of *Acteon* in having a smooth columella (without columellar folds) and the shell surface sculptured with spiral cords (punctated spiral grooves are present in *Acteon* species).

Significance of the New Species in the Context of Present Knowledge of the Geographic Distribution of the Genus

The new species here described represents the northernmost record for *Neactaeonina*, a genus thus far known only from Antarctica, New Zealand, and the Scotia Arc islands. However, the present finding does not necessarily imply the extension of the distribution of the genus to a different biogeographic realm: in fact, the localities where the new species was collected correspond to an area affected by the so-called sub-Antarctic waters, influenced by the Malvinas oceanic current, which is a branch of the West Wind Drift extending northwards, along the outer margin of the Argentine shelf, up to 23°S (off Brazil) (Boltovskoy et al., 1999). In fact, the benthic assemblages occurring at the sites where ***Neactaeonina argentina***, n. sp., was collected include several well known sub-Antarctic taxa: the bivalves *Zygochlamys patagonica* (King, 1832), *Limatula pygmaea* (Philippi, 1845), *Hiatella meridionalis* d'Orbigny, 1846; the gastropods *Calyptraea pileolus* d'Orbigny, 1841, *Fusitriton magellanicus* (Röding, 1798), *Odontocymbiola magellanica* (Gmelin, 1791), *Adelomelon ancilla* ([Lightfoot], 1786); the sponges *Tedania massa* Ridley & Dendy, 1886, *Tedania mucosa* Thiele, 1905, *Hymedesmia antarctica* Hentschel, 1914, *Iophon proximum* (Ridley, 1881), *Phorbas leptochela* (Hentschel, 1914), *Tedania charcoti* Thiele, 1905, *Isodictya verrucosa* (Topsent, 1913), *Haliclona kerguelensis* (Hentschel, 1914); the echinoderms genera *Labidiaster* Lütken, 1871, *Odontaster* Verrill, 1880, *Acodontaster* Verrill, 1899, *Porania* Gray, 1840, *Ophiacanta* Müller & Troschel, 1842, *Gorgonocephalus* Leach, 1815, *Sterechinus* Koehler, 1901; and the cnidarian genera *Alcyonium* Linnaeus, 1758, *Thouarella* Gray, 1870, and *Actinostola* Verrill, 1883 (Bertolino et al., 2007; Roux et al., 2005; Schejter et al., 2006; Schejter unpubl. data). The above information confirms that *Neactaeonina* is a genus clearly associated with the sub-Antarctic and Antarctic waters.

Species Erroneously Reported under *Neactaeonina*

Marcus (1974) reported under *Neactaeonina, Acteon (Actaeonina) chariis* Watson, 1881, a living species from the Azores. The only argument for this generic placement was that "The genus *Acteonina* d'Orbigny, 1850, was erected for a fossil species". Thiele (1912: 220) and Zilch (1959–60: 14) retained the use of *Acteonina* for fossil shells. The radula and soft part anatomy of *Acteon (Actaeonina) chariis* are unknown; however, several shell characters, such as the presence of a relatively high spire, the sinuous ("hair-like") growth lines, and the protoconch "extremely blunt", strikingly different from the condition present in the sub-Antarctic and Antactic species of *Neactaeonina*. Mikkelsen (1995), Malaquias (2001), and Cervera et al. (2004) retained this species under *Actaeonina*.

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ROBUST SHELL PHENOTYPE IS A LOCAL RESPONSE TO STREAM SIZE IN THE GENUS *PLEUROCERA* (RAFINESQUE, 1818)

Robert T. Dillon, Jr.

Department of Biology, College of Charleston, Charleston, South Carolina 29424 USA; dillonr@cofc.edu

ABSTRACT

Although local correlations between shell phenotype and stream size have often been documented in freshwater mollusks, the species- and even genus-level taxonomy of pleurocerid snails has historically been based almost entirely on aspects of the shell. Here I test the hypothesis that lightly shelled pleurocerid populations inhabiting smaller rivers in east Tennessee and north Georgia, variously assigned to the genus *Goniobasis* or *Elimia*, may be local variants of heavily shelled *Pleurocera* populations downstream. Populations of the nominal species *Goniobasis* ("*Elimia*") *acutocarinata*, *G. clavaeformis*, and *Pleurocera unciale* were sampled from the Powell, Little, and Hiwassee subdrainages of the Tennessee River, and populations nominally *Goniobasis carinifera* and *Pleurocera vestita* sampled from the Coahulla subdrainage of the Mobile Basin. A population of *Goniobasis simplex* was sampled from each of the four subdrainages to calibrate expected levels of genetic divergence. Gene frequencies at ten polymorphic allozyme-encoding loci (15 populations, 30 individuals per population) revealed that each population of *Pleurocera* was more closely related to its local populations of *Goniobasis* (or "*Elimia*") than to any other population of *Pleurocera*. All nine populations identified as *G. acutocarinata*, *G. clavaeformis*, and *P. unciale* appear to be conspecific, their minimum genetic identity of 0.771 much greater than the 0.356 minimum identity among the four *G. simplex* controls. The specific relationship between the nine Tennessee populations and populations of *G. carinifera* and *P. vestita* from the Mobile Basin is ambiguous, with identities ranging down to 0.284. This larger set of 11 populations is here referred to as the *carinifera* group. Evidence that intraspecific variation in shell morphology has risen to the level of the genus suggests that *Goniobasis*, *Elimia*, and several other generic nomina be subsumed under *Pleurocera* (Rafinesque, 1818).

Key words: Shell morphology, ecophenotypic plasticity, genetic divergence, allozyme electrophoresis, freshwater gastropods, Pleuroceridae, *Goniobasis*, *Elimia*.

INTRODUCTION

Correlations between stream size and the shell phenotype of the freshwater mollusk populations that inhabit them have been the object of research interest for almost 100 years (Adams, 1915; Ortmann, 1920). The phenomenon was well documented by Calvin Goodrich, who published a series of eight papers on phenotypic variation in pleurocerid snails between 1934 and 1941. Relationships between stream size, current, or substrata and shell phenotype have more recently been demonstrated in the pulmonate gastropod *Lymnaea* (Lam & Calow, 1988), unionid mussels (Watters, 1994), the pleurocerids *Semisulcospira* (Urabe, 1998, 2000) and *Lithasia* (Minton et al., 2008), and the pulmonate limpet *Ferrissia* (Dillon & Herman, 2009).

Goodrich (1934, 1935, 1937) observed that populations of pleurocerid snails often vary in shell shape and sculpture from slender and more angulate in smaller tributaries to more robust or "obese" in larger rivers. He attributed the greater frequency of broader, more robust shells in downstream populations to physical disturbance or water current. Goodrich was influenced by the work of Wiebe (1926) who suggested that the "relatively great obesity" of the pleurocerid *Goniobasis* (or "*Elimia*") *livescens* on exposed shores of Lake Erie originated from the adaptive value of a large foot in areas of heavy wave action. The broader shell might be a secondary consequence of a larger foot. Goodrich suggested that this phenomenon might generalize to rivers and streams, observing that "an ecological analogy would appear to exist between the exposed situations of Lake Erie, inhabited by obese *G. livescens*, and the rapid and sometimes tumultuous southern creeks and river headwaters where other obese pleurocerids have their habitats."

More recently, significant research interest has been directed towards the effects of predation on freshwater gastropod shell morphology (DeWitt et al., 2000; Rundle et al., 2004; Holomuzki & Biggs, 2006; Lakowitz et al., 2008; Hoverman & Relyea, 2009). Krist (2002) reared *G. livescens* in effluent from crayfish feeding on conspecific snails and documented a significant narrowing of the body whorl compared to controls. Although such experiments have not typically been designed to test for the effects of stream size or current directly, any increase in the size or abundance of crushing predators in the downstream reaches of a river might be expected to translate into more robust shell morphology in their freshwater gastropod prey.

In many of the situations examined by Goodrich, earlier taxonomists had described downstream morphological variants of pleurocerid populations as distinct species, which Goodrich subsequently synonymized. For example, *Goniobasis* (or "*Elimia*") *clavaeformis* (Lea, 1841) is a common inhabitant of mid-sized rivers in east Tennessee and southwest Virginia, typically bearing a moderately robust shell with rounded body whorls. Headwater streams in these same regions are inhabited by *Goniobasis* (or "*Elimia*") populations with thinner, more highly spired shells bearing carinae, previously referred to *Goniobasis acutocarinata* (Lea, 1841). Goodrich (1940) observed that populations in streams of intermediate size bore shells of intermediate robustness between the two types, synonymizing the nomen "*acutocarinata*" under *G. clavaeformis*.

Larger streams in southwest Virginia, such as the Clinch, Powell, and Holston rivers, are inhabited by pleurocerid populations that were identified by Goodrich (1940) as *Pleurocera unciale* (= *uncialis*, Haldeman 1841). These populations have shells that are heavier and more robust than typical *Goniobasis* populations, their whorls typically marked with prominent anterior angulation and their anterior apertures at least slightly flared. Dillon & Robinson (2007a, b) have reported evidence from gene frequencies at allozyme-encoding loci suggesting that the shell morphology demonstrated by populations identified as "*Pleurocera unciale*" in the upper Powell River might represent downstream morphological variants of *G. clavaeformis*, just as the "*acutocarinata*" morphology represents the upstream.

Through extensive application of over 30 years, the technique of allozyme electrophoresis has proven a valuable tool for measuring genetic diversity within and among populations of pleurocerid snails (Chambers, 1978; Dillon & Davis, 1980; Bianchi et al., 1994; Dillon & Lydeard, 1998). Levels of genetic divergence are best understood in *Goniobasis proxima*, from which dozens of populations have been studied in five states (Dillon, 1984; Stiven & Kreiser, 1994), with calibration against breeding data (Dillon, 1986, 1988a), cytogenetics (Dillon, 1991) and mitochondrial sequence divergence (Dillon & Frankis, 2004; Dillon & Robinson, 2009). Genetic divergence at allozyme-encoding loci has also been well studied among populations of *Goniobasis catenaria*, ranging from North Carolina through Georgia (Dillon & Reed, 2002; Dillon & Robinson, in press).

Here I extend the survey of Dillon & Robinson (2007a, b) from southwest Virginia through east Tennessee and into north Georgia. I select four subdrainages – the Powell, Little, Conasauga, and Cohutta rivers – and within each of these subdrainages sample populations displaying the *G. acutocarinata – G. clavaeformis – P. unciale* morphology along a gradient of stream size. Because of taxonomic uncertainty in populations bearing shells of this general form, I also sample reference populations of *G. simplex*, a widespread and easily recognized species, to calibrate expected levels of genetic divergence. I then estimate genetic divergence among all 15 populations at ten polymorphic loci using allozyme electrophoresis. If populations of the more robustly shelled *Pleurocera* type in the larger rivers are more genetically similar to their local populations of *Goniobasis* than they are to each other, the hypothesis of Dillon & Robinson (2007a, b) will be confirmed.

METHODS

Taxonomy

No consensus regarding the taxonomy of the Pleuroceridae has emerged at any time throughout the history of North American malacology. The taxonomy of Goodrich (1940) is used throughout the present work for consistency, deferring resolution of the issues raised to an Appendix.

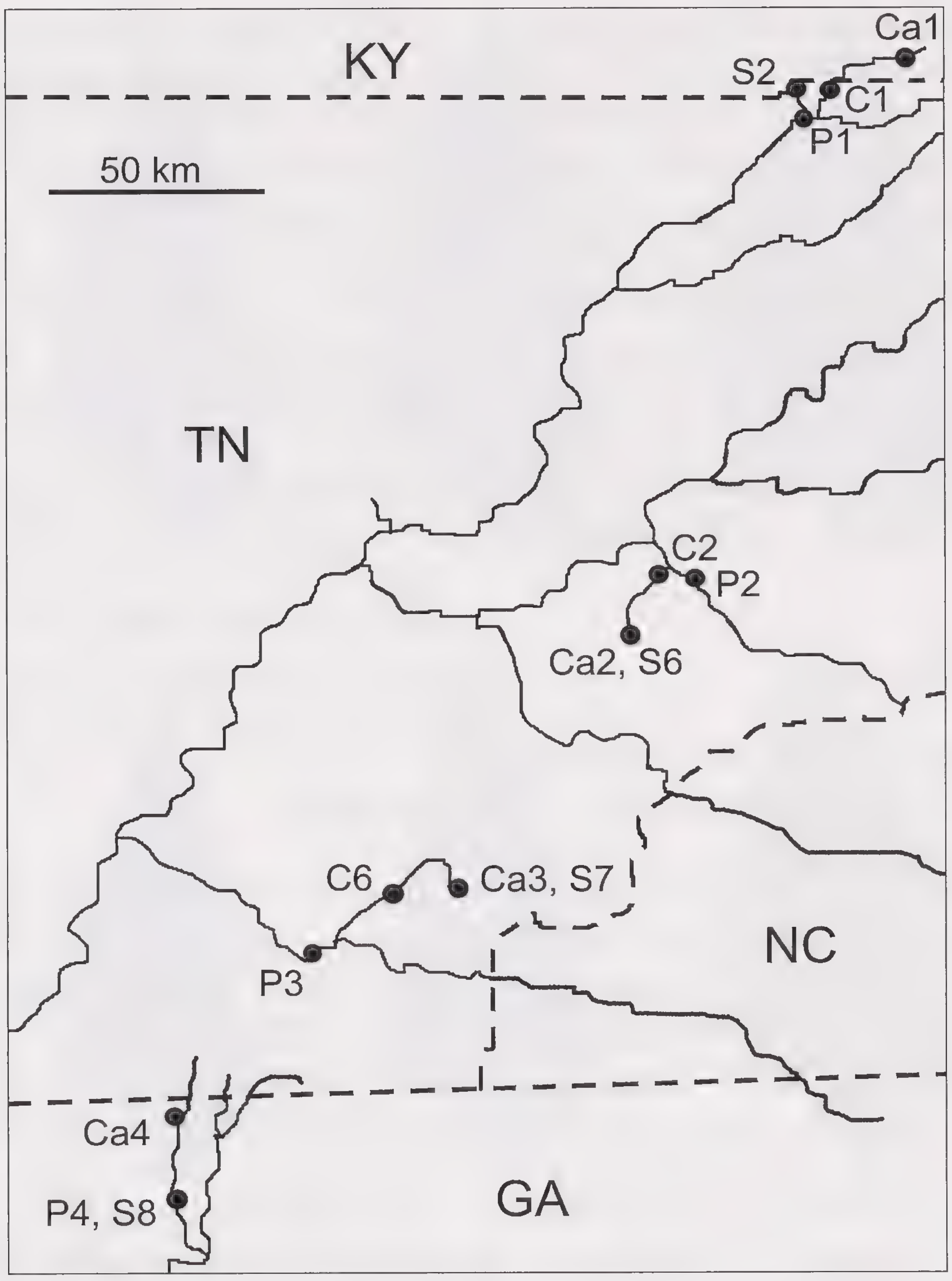


FIG. 1. River drainages in southwest Virginia, east Tennessee, and north Georgia, showing sample sites for all pleurocerid populations sampled in this study.

Populations Studied

At least 30 individual snails were sampled from 15 pleurocerid populations as follows. CA1 – *Goniobasis "acutocarinata"* from Indian Creek at Co. 724 bridge (Ewing), Lee County, Virginia (36.6374°N, 83.4316°W). This was site VG055 of Dillon & Robinson (2007a). C1 – *Goniobasis clavaeformis* from Indian Creek at Co. 684 bridge (Kesterson Mill), Lee County, Virginia (36.6283°N, 83.5019°W). This was Site 1 of Dillon & Robinson (2007a). P1 – *Pleurocera unciale* from the Powell River by River Road, 4 km S of Harrogate, Claiborne Co, Tennessee (36.5544°N, 83.6091°W). S2 – *Goniobasis simplex* from the head of Gap Creek in Cumberland Gap, Claiborne County, Tennessee (36.6001°N, 83.6681°W). This was Site 2 of Dillon & Robinson (2007a). CA2 – *Goniobasis "acutocarinata"* from Pistol Creek in the Courthouse Park, Maryville, Blount County, Tennessee (35.7535°N, 83.9711°W). S6 – *Goniobasis simplex* from the same location. C2 – *Goniobasis clavaeformis* from Pistol Creek below Williams Mill Dam, 5 km N of Maryville, Blount County, Tennessee (35.8147°N, 83.9424°W). P2 – *Pleurocera unciale* from Little River at the US 411 bridge, 6 km E of Maryville, Blount County, Tennessee (35.7856°N; 83.8841°W). CA3 – *Goniobasis "acutocarinata"* from Lick Creek at Shoal Creek Road, 3 km NW of Jalapa, Monroe County, Tennessee (35.3583°N, 84.3881°W). S7 – *Goniobasis simplex* from the same location. C6 – *Goniobasis clavaeformis* from Conasauga Creek at the Co. 879 bridge, 5 km E of Etowah, McMinn County, Tennessee (35.3236°N, 84.4791°W). P3 – *Pleurocera unciale* from the Hiwassee River at the boat ramp 4 km N of Benton, Polk County, Tennessee (35.2035°N, 84.6531°W). The robustness of the shells borne by this population may rise to fit *Pleurocera curtum*, in the concept of Goodrich

(1928). CA4 – *Goniobasis carinifera* from the spring at the Cohutta Fisheries Center Public Park, Whitfield County, Georgia (34.9731°N, 84.9505°W). P4 – *Pleurocera vestita* from Coahulla Creek at the GA 2 bridge (Prater Mill), Whitfield County, Georgia (34.8968°N, 84.9202°W). S8 – *Goniobasis simplex* from the same location. A map locating the collection sites for all populations is given in Figure 1, and example shells are shown in Figure 2. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia (pending).

Allozyme Electrophoresis

Snails were returned alive to the laboratory, where they were cracked and frozen in tris tissue buffer for electrophoretic analysis. Techniques and apparatus for horizontal starch gel electrophoretic resolution of allozyme variation in homogenates of molluscan tissues are detailed in Dillon (1992), along with recipes for all the buffers and stains employed here. Dillon & Robinson (2007a) initially screened five individuals from four nominal pleurocerid species for scorable polymorphisms on four buffer systems staining for 19 enzymes. The enzymes tested were alcohol dehydrogenases (ethanol, hexanol, octanol), sorbitol dehydrogenase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, xanthine dehydrogenase, octopine dehydrogenase, superoxide dismutase, aspartate aminotransferase, phosphoglucomutase, esterases (napthyl acetate, napthyl proprionate), phosphatases (acid, alkaline), leucine aminopeptidase, mannose phosphate isomerase, and glucose phosphate isomerase. Ultimately, bands interpretable as the products of codominant genes segregating in Mendelian fashion at the loci using nine enzyme stains were resolved as detailed below.

The Tris Cit 6 buffer (buffer XIII of Shaw & Prasad, 1970) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), octopine dehydrogenase (OPDH), and isocitrate dehydrogenase (two loci, the cathodal IDHF and the anodal IDHS). A Poulik (1957) discontinuous buffer system was employed for glucose-phosphate isomerase (GPI), and octopine dehydrogenase (a second time). The TEB8 buffer system (buffer III of Shaw & Prasad, 1970) was used to analyze phosphoglucomutase (PGM – the strong, fast locus only), xanthine dehydrogenase (XDH), and mannose phosphate isomerase (MPI). A TEB9.1 buffer (Dillon & Davis, 1980) was used for octanol dehydrogenase (OLDH), esterases (EST1 – the strong, slow locus only) and xanthine dehydrogenase (a second time).

Mendelian inheritance of allozyme phenotype has been confirmed for GPI, OPDH, and EST1 by Dillon (1986) and for 6PGD by Chambers (1980). Putative allelic designations for each zone of allozyme activity were assigned by setting the population of *G. simplex* from its type locality as a standard (population S5 of Dillon & Robinson, 2007a). The putative allele encoding the most common allozyme at population S5 was designated “100”, and all other alleles were named by the mobility of their allozymes (in millimeters) relative to this standard.

Analysis

Gene frequencies and mean direct-count heterozygosities (the unbiased estimate of Nei, 1978) were calculated using Biosys version 1.7 (Swofford & Selander, 1981). Because large numbers of alleles were resolved at some loci, sample sizes dictated that genotypes be pooled into three classes before testing for Hardy-Weinberg equilibrium: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes. Yates-corrected chi-square statistics were then employed for this purpose. I calculated matrices of Nei’s (1978) unbiased genetic identity and distance, as well as Cavalli-Sforza & Edwards (1967) chord distance. As chord distances are Pythagorean in Euclidean space (Wright, 1978), they were used as the basis for a neighbor-joining tree (Phylip v3.65 program NEIGHBOR, Felsenstein, 2004).

RESULTS

Putative gene frequencies at the ten allozyme-encoding loci examined are reported for 15 pleurocerid populations in Table 1, together with mean direct-count heterozygosities. Of the 10 x 15 = 150 loci examined, 47 were polymorphic by the 95% criterion. Genotype frequencies at three of these 47 loci were significantly different from Hardy-Weinberg expectation by chi-square tests at the nominal 0.05 level, a result clearly attributable to type I statistical error.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown in Figure 3, together with the results of a neigh-

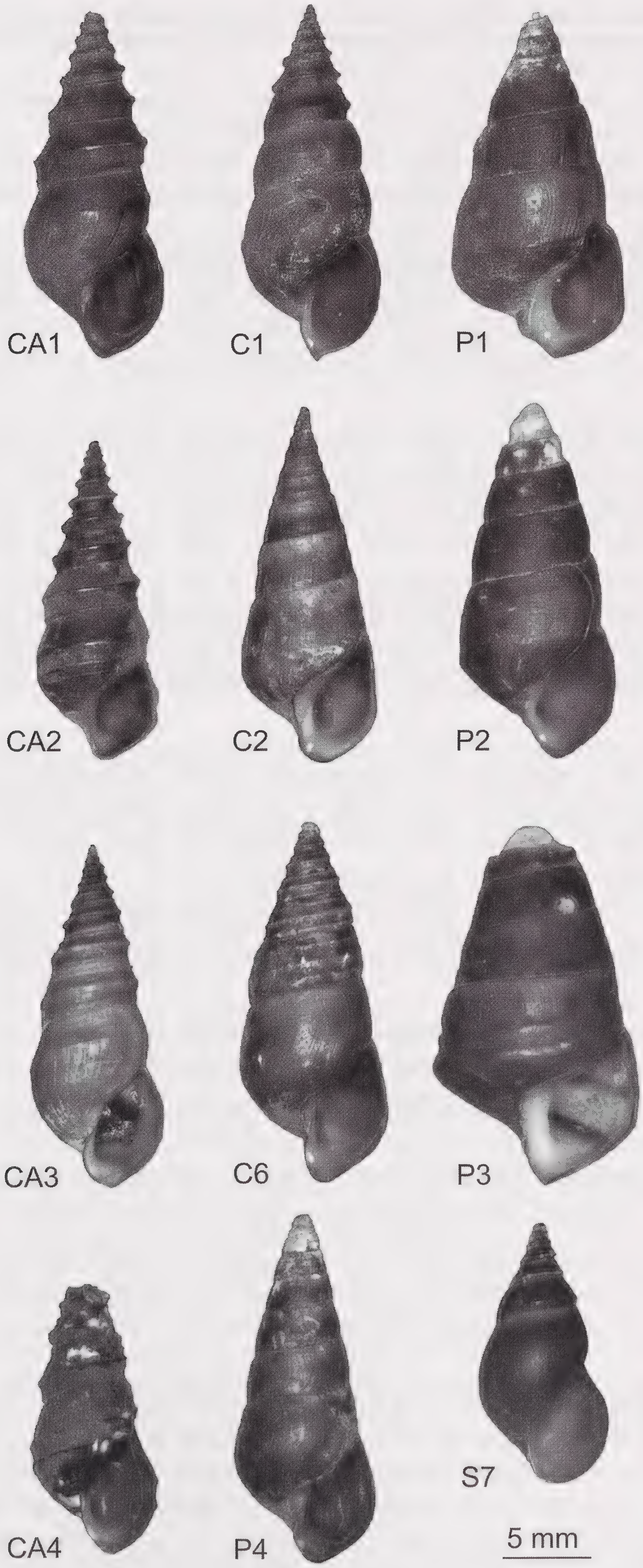


FIG. 2. Example shells from most of the pleurocerid populations studied. Only a single representative shell of *G. simplex* is figured (S7); shells from the other three *simplex* populations were similar.

TABLE 1. Gene frequencies and average (direct count) heterozygosity (H) over ten polymorphic enzyme loci in 15 pleurocerid populations from 4 subdrainages in the southern Appalachians.

	Powell			Little			Hiwassee			Coahulla		*G. simplex*			
Allele	Ca1	C1	P1	Ca2	C2	P2	Ca3	C6	P3	Ca4	P4	S2	S6	S7	S8
GPI															
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.065
99	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000
98	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.935
MPI															
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.950	1.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.933	0.984	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.016	0.000	0.000	0.000	0.000
93	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.984	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
87	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EST1															
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.032
101	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.933	0.968
99	0.000	0.000	0.000	0.015	0.015	0.000	0.603	0.258	0.017	0.323	0.917	0.000	0.000	0.000	0.000
98	1.000	1.000	1.000	0.985	0.971	0.941	0.397	0.742	0.983	0.677	0.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.015	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PGD															
106	0.000	0.000	0.107	0.000	0.000	0.059	0.000	0.017	0.067	0.000	0.034	0.000	0.000	0.000	0.000
103	0.732	0.973	0.571	0.530	0.576	0.809	0.692	0.883	0.667	0.183	0.172	0.000	0.000	0.000	0.000
100	0.268	0.027	0.321	0.470	0.424	0.132	0.308	0.100	0.267	0.817	0.793	1.000	1.000	1.000	1.000
PGM															
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.403
102	0.887	0.945	1.000	0.029	0.074	0.559	0.000	0.000	0.383	0.952	0.783	0.094	0.054	0.000	0.597
101	0.000	0.000	0.000	0.000	0.000	0.000	0.362	0.733	0.000	0.048	0.217	0.000	0.000	0.000	0.000
100	0.113	0.055	0.000	0.971	0.926	0.441	0.638	0.267	0.517	0.000	0.000	0.906	0.000	1.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.946	0.000	0.000
OPDH															
120	0.000	0.000	0.145	0.000	0.044	0.206	0.103	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000
119	0.000	0.000	0.000	0.000	0.000	0.147	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
118	0.774	0.675	0.677	1.000	0.956	0.574	0.776	0.516	0.867	0.000	0.000	0.000	0.000	0.000	0.000
116	0.226	0.325	0.113	0.000	0.000	0.074	0.121	0.242	0.067	0.000	0.000	0.000	0.000	0.000	0.000
112	0.000	0.000	0.065	0.000	0.000	0.000	0.000	0.242	0.017	0.484	1.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.516	0.000	0.000	0.000	0.000	0.000

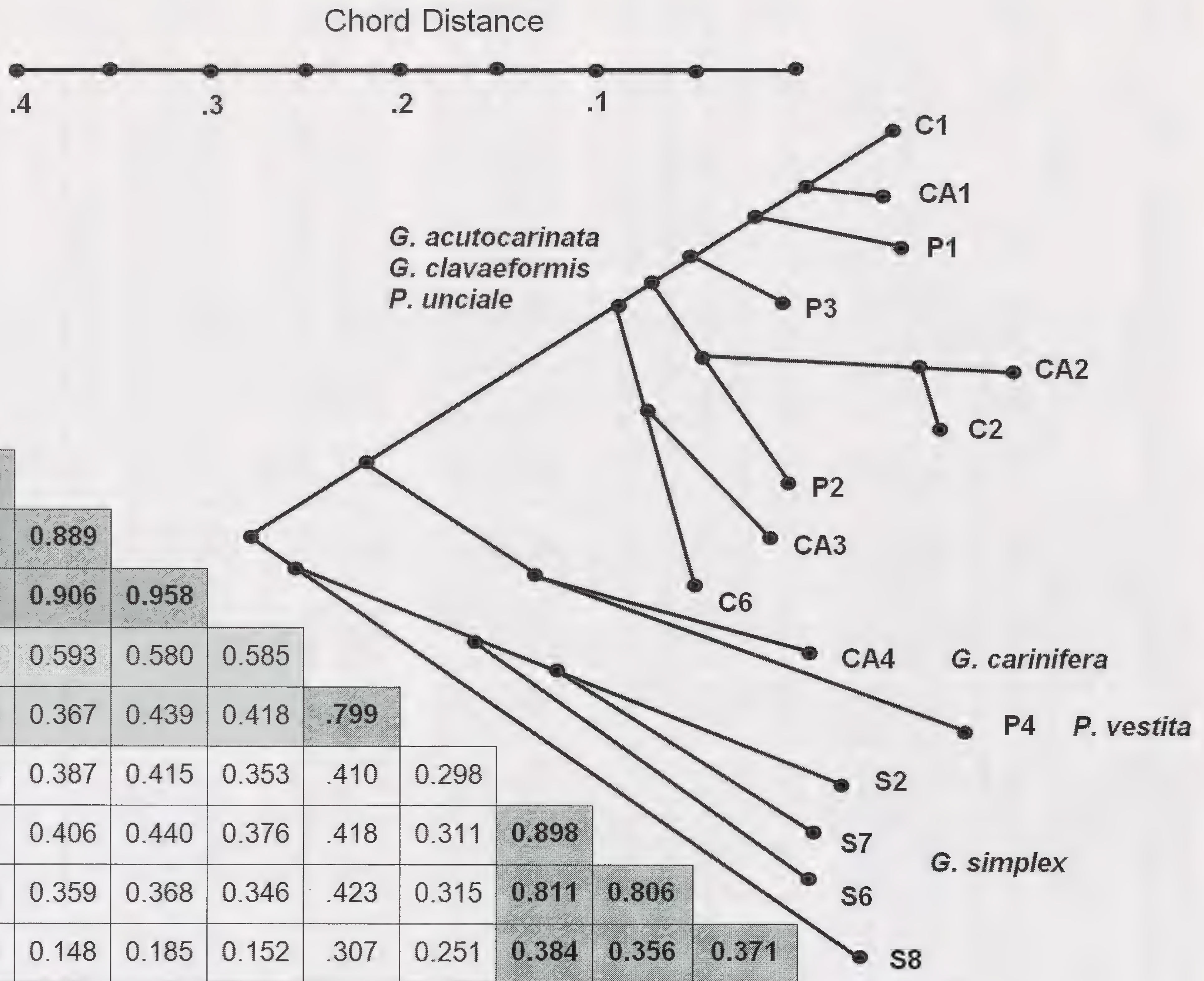
(continues)

(continued)

	Powell			Little			Hiwassee			Coahulla		*G. simplex*			
Allele	Ca1	C1	P1	Ca2	C2	P2	Ca3	C6	P3	Ca4	P4	S2	S6	S7	S8
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	1.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.953	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.946	0.000	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
IDHF															
107	0.000	0.000	0.000	1.000	0.941	0.545	0.000	0.083	0.017	0.000	0.000	0.000	0.000	0.000	0.000
102	1.000	1.000	1.000	0.000	0.059	0.455	1.000	0.917	0.950	1.000	0.645	0.000	0.000	0.000	0.903
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	0.000
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.355	0.000	0.000	0.000	0.000
IDHS															
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.048	1.000	1.000	1.000	0.033
98	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.952	0.000	0.000	0.000	0.000
97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.967
OLDH															
106	0.083	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.683
100	0.917	0.947	1.000	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	0.969	1.000	1.000	0.317
XDH															
101	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.781	1.000	1.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.219	0.000	0.000	0.000
H	0.100	0.065	0.102	0.066	0.102	0.181	0.168	0.147	0.140	0.104	0.106	0.059	0.022	0.023	0.098

bor-joining analysis based on Cavalli-Sforza & Edwards Chord distances. The four control populations of *G. simplex* clustered together, as expected, with genetic identities ranging from 0.898 to 0.356. Within the larger group of 11 study populations, clustering was primarily by region, independent of putative taxonomy. The three populations from the Powell River subdrainage (C1, CA1 and P1) were most similar to each other genetically, as were the three populations from the Little River subdrainage (C2, CA2, and P2) and the two populations from the Coahulla (CA4 and P4). Population P3 was somewhat separated from the other two populations of the Hiwassee subdrainage (C6 and CA3), being intermediate between the Powell subdrainage group and the Little subdrainage group, but again, independent of putative taxonomy.

Calibrated against the *G. simplex* standard, all populations previously assigned to *G. acutocarinata*, *G. clavaeformis*, and *P. unciale* are clearly attributable to a single species of highly variable shell morphology. The genetic identities among this set of nine populations ranged down only to 0.771, as against the 0.356 among the *G. simplex* controls. The two north Georgia populations, previously assigned to *G. carinifera* and *P. vestita*, showed genetic identities ranging from 0.667 down to 0.284 with the larger set of nine Tennessee populations, leaving their specific relationships ambiguous. The label "*carinifera* group" would be most appropriate to describe this set of 11 populations together, since *"Melania" carinifera* (Lamarck 1822) is the oldest of the many names available.



	C1	CA1	P1	P3	CA2	C2	P2	CA3	C6	CA4	P4	S2	S7	S6
CA1	0.994													
P1	0.983	0.995												
P3	0.957	0.975	0.961											
CA2	0.771	0.801	0.780	0.875										
C2	0.794	0.821	0.801	0.891	1.00									
P2	0.942	0.946	0.938	0.958	0.923	0.939								
CA3	0.871	0.889	0.869	0.947	0.830	0.845	0.889							
C6	0.905	0.905	0.886	0.943	0.808	0.826	0.906	0.958						
CA4	0.644	0.667	0.691	0.625	0.467	0.482	0.593	0.580	0.585					
P4	0.401	0.418	0.446	0.391	0.284	0.294	0.367	0.439	0.418	.799				
S2	0.308	0.344	0.347	0.393	0.438	0.436	0.387	0.415	0.353	.410	0.298			
S7	0.318	0.354	0.356	0.413	0.463	0.461	0.406	0.440	0.376	.418	0.311	0.898		
S6	0.317	0.347	0.361	0.358	0.362	0.363	0.359	0.368	0.346	.423	0.315	0.811	0.806	
S8	0.204	0.231	0.247	0.198	.093	0.098	0.148	0.185	0.152	.307	0.251	0.384	0.356	0.371

FIG. 3. Nei's (1978) unbiased genetic identities are shown below the diagonal, with conspecific values shaded. Paler shading marks values comparing *clavaeformis* with the other members of the *carinifera* complex. Above the diagonal is a neighbor-joining tree based on Cavalli-Sforza & Edwards (1967) genetic distances.

DISCUSSION

The results depicted in Figure 3 confirm and generalize the observations of Dillon & Robinson (2007a, b). Independently, in four different subdrainages, populations of pleurocerid snails apparently demonstrate a more robust shell phenotype as stream size increases. In headwater streams of the Tennessee system, such populations have been identified as *G. acutocarinata*, in middle reaches they have been called *G. clavaeformis*, and in larger rivers they have been called *Pleurocera unciale,* or perhaps by some authors *P. curtum*. In headwaters of the Alabama/Coosa system, populations of this group have been identified as *G. carinifera*, and in middle reaches as *Pleurocera vestita*.

Populations of pleurocerids bearing shells in the *acutocarinata – clavaeformis – unciale* gradient of morphology are the most common freshwater gastropods in east Tennessee, certainly ranging from the Clinch and Holston subdrainages of southwest Virginia through the western French Broad and Little Tennessee drainages to the vicinity of Chattanooga, probably further west. The names *clavaeformis* (Lea, March 1841: 12) and *acutocarinata* (Lea, March 1841: 14) and would have priority over *unciale* (Haldeman, Oct. 1841) to refer to this single, highly variable species, and I here select the first name as first reviser.

It should be emphasized that stream size *per se*, as it might be measured by catchment area or discharge volume, is almost certainly not the variable to which these pleurocerid populations are responding. Water chemistry, temperature, depth, current, substrate, primary and secondary productivity, allochthanous input, and the entire communities of producers, herbivores and carnivores are all expected to vary significantly down a river gradient. To determine which of these many environmental and ecological factors might most directly affect the shell morphology of resident pleurocerid populations would require extensive experimentation.

The proportion of the variation here documented in shell phenotype that might have an additive genetic basis also cannot be determined at present. *Goniobasis* populations may not be panmictic, even at a scale of meters (Dillon, 1988b). Populations CA3 and C6, for example, separated by approximately 12 km, showed significant differences at three loci (EST1, PGM and OPDH), and populations C1 and CA1, separated by approximately 6 km, showed a significant difference at 6PGD (Table 1).

However, a great many controlled studies involving other freshwater gastropods have returned evidence that the additive genetic component of shell robustness may be low. The literature on inducible shell defenses in pulmonate snails is especially rich, with striking and often rapid ecophenotypic responses to predation in shell thickness, shell width, and aperture shape documented in physids (DeWitt et al., 2000; Langerhans & DeWitt, 2002), lymnaeids (Rundle et al., 2004; Lakowitz et al., 2008) and planorbids (Hoverman & Relyea, 2007, 2009). The laboratory experiments of Urabe (1998, 2000) returned evidence that both shell shape and shell sculpture are ecophenotypically plastic responses to current and substrate in the Japanese pleurocerid *Semisulcospira*.

The pleurocerid genera *Pleurocera* and *Goniobasis* or *Elimia* have historically been distinguished by a flare or notch in the anterior aperture of the former, a weak and unreliable character. Thorough anatomical investigations have returned no evidence of any significant difference between the two genera (Dazo, 1965; Strong, 2005), nor have mtDNA sequence studies (Sides, 2005). The evidence offered here, that interpopulation variation in shell morphology can rise to the level of the genus, suggests that *Pleurocera*, *Goniobasis* and *Elimia* be combined. See also the Appendix.

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APPENDIX

The taxonomy of the Pleuroceridae was entirely unstable throughout the nineteenth century, hundreds of species being described and dozens of genera being proposed to contain them (Tryon, 1873). Modern workers have generally recognized seven or eight North American genera, but have often selected different nineteenth-century names to call them. Walker (1918) preferred *Io*, *Lithasia*, *Gyrotoma*, *Eurycaelon*, *Anculosa*, *Goniobasis* and *Pleurocera*. Goodrich (1924, 1940) split *Nitocris* from *Anculosa* but otherwise followed closely: *Io, Lithasia, Gyrotoma, Eurycaelon, Anculosa, Nitocris, Goniobasis* and *Pleurocera*. Morrison (1954) considered that Rafinesque's nomen "*Pleurocera*" was more correctly applied to the group that Walker termed "*Lithasia*," and that the Rafinesque nomen "*Oxytrema*" was better applied to a combination of Walker's *Pleurocera* and *Goniobasis*. He also preferred *Leptoxis* over *Anculosa*, and split the genus referred to as "*Nitocris*" by Goodrich into *Mudalia* and *Anaplocamus*. The result again yielded eight genera: *Io, Pleurocera, Gyrotoma, Eurycaelon, Leptoxis, Mudalia, Anaplocamus*, and *Oxytrema*. Burch & Tottenham (1980) returned to the Walker/Goodrich concept of *Pleurocera*, anticipating Opinion 1195 of the ICZN (Melville, 1981). Burch subsumed *Eurycaelon*, *Mudalia*,and *Anaplocamus* of Morrison under the single genus *Leptoxis*, preferred *Elimia* over *Goniobasis*, and split out the western *Juga*, yielding: *Io, Lithasia, Gyrotoma, Leptoxis, Juga, Elimia*, and *Pleurocera*.

The research results presented in the main body of the present work demonstrate that the shell characters by which Walker, Goodrich, and Burch distinguished *Pleurocera* from either *Goniobasis* or *Elimia* do not rise to the level of the genus. Hence, these two groups should be combined, as preferred by Morrison. But following Opinion 1195, the oldest available name of the combined group is not *Oxytrema*, but rather *Pleurocera*, as follows:

Family Pleuroceridae Fischer, 1885
Genus *Pleurocera* Rafinesque, 1818

Pleurocera Rafinesque, 1818: 355. Type species, *Pleurocerus acutus* Rafinesque in Blainville, 1824. Opinion 1195 of the ICZN (Melville, 1981).

Oxytrema Rafinesque, 1819: 423, genus without species. Type species, *P. acutus* Rafinesque, by subsequent monotypy by Blainville (Morrison, 1954: 360).

Ceriphasia Swainson, 1840: 204. Type species, *C. sulcata* Swainson, 1840, by monotypy.

Telescopella Gray, 1847: 153. Type species, *Melania undulata* Say, 1829, by original designation.

Elimia H. Adams & A. Adams, 1854: 300. Type species *Melania acutocarinata* Lea, 1841, by subsequent designation of Pilsbry & Rhoads (1896).

Melasma H. Adams & A. Adams, 1854: 300. Type species *Melania laqueata* Say, 1829, by subsequent designation of Baker (1963).

Strephobasis Lea, 1861: 96. Type species *Melania plena* Anthony, 1854, by subsequent designation of Pilsbry & Rhoads (1896).

Trypanostoma Lea, 1862: 169. Type species *Melania canaliculata* Say, 1821, by original designation.

Goniobasis Lea, 1862: 262. Type species, *G. osculata* Lea, 1862, = *Melania olivula* Conrad, 1834, by subsequent designation of Hannibal, 1912.

Macrolimen Lea, 1863: 220. Type species *Melania showalterii* Lea, 1862, by monotypy.

Strepoma Haldeman, 1863: 274, *ex* Rafinesque ms. Type species, *Melania canaliculata* Say, 1821.

Diagnosis: A genus in the freshwater Cerithiacean family Pleuroceridae with elongate-conic or cylindrical shells, often carinate or costate but not spinose or (ordinarily) pustulate. Aperture may be notched anteriorly, but never posteriorly. Dioecious, sexually reproducing. Differing from the similar *Juga* by the absence of a seminal receptacle (Strong & Frest, 2007).

Description: See Strong (2005) for a thorough description of *P. acuta* and *E. livescens*. All differences noted between those two taxa are here considered significant only at the specific level, not at the level of genus.

Distribution and Habitat: Found in freshwater habitats through much of eastern North America, from northern Florida to southern Ontario, west to Texas and Minnesota. Primarily an inhabitant of rivers and streams, but may also be abundant in cooler, well-oxygenated lentic environments.

Phylogenetic Relationships: Dillon & Robinson (2009) suggested that populations of *Pleurocera* (as here more broadly defined) inhabiting the southern Appalachians may have undergone little morphological evolution since the Mesozoic Era, or possibly even the Paleozoic. Molecular evolution seems to have continued unabated, however, yielding intrapopulation sequence divergence up to 21.9% for some mitochondrial genes. It is perhaps unsurprising, therefore, that larger molecular phylogenetic surveys of the North American Pleuroceridae published thus far have returned mixed and contradictory results (Holznagel & Lydeard, 2000; Lydeard et al., 2002; Ó Foighil et al., 2009). Molecular confirmation of the genus *Pleurocera* as proposed here may await data from some more slowly-evolving markers, as yet unidentified.

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A SURVEY OF VITRINID LAND SNAILS (GASTROPODA: PULMONATA: LIMACOIDEA)

Folco Giusti*, Viviana Fiorentino, Andrea Benocci & Giuseppe Manganelli

Dipartimento di Scienze Ambientali, Università di Siena, Via Mattioli 4, I-53100 Siena, Italy

ABSTRACT

There is general agreement that the vitrinids, a mainly Palaearctic group of semislugs, are higher stylommatophorans, but uncertainty still exists about their relationships. We therefore conducted a phylogenetic study based on morphological characters to determine relationships between genus-group taxa and to test monophyly and revise current taxonomy. Eighteen genus-group taxa – those listed as valid in the recent literature plus the new genus *Azorivitrina* – were considered using an exemplar approach, with individual species as terminals to avoid *a priori* assumption of monophyly. At least two species, one of which was the type species, were examined when possible. Phylogenetic analyses were performed using different outgroups consisting of a selection of western Palaearctic limacoidean genera (data partition I, DPI: outgroup consisting of *Euconulus*, *Deroceras*, *Oxychilus*, *Tandonia*, *Vitrea* and *Troglaegopis*; DPII: outgroup *Euconulus*, *Oxychilus*, *Vitrea* and *Troglaegopis*; DPIII: outgroup *Euconulus* and *Oxychilus*). Different approaches were used: parsimony analysis (MP: using PAUP* 4.0; AP: TNT software) and bayesian inference (BI: using MRBAYES). Phylogeny analysis generally had low resolution. The parsimony analysis using implied weights (TNT software) provided the best results (AP found 11 supported clades, MP 11 and BI 8). It did not support monophyly of most genus-group taxa or the monophyletic groups found by Hausdorf (1995, 2002) and Alonso et al. (2000), or the subfamilies and tribes established by Schileyko (1986, 2003), or the family Vitrinidae when the outgroup consisted of a large selection of limacoidean taxa. Support was only found for *Canarivitrina*, *Guerrina*, *Oligolimax* and *Vitrinobrachium* and some support for *Arabivitrina* and *Azorivitrina*. On the contrary, no support was found for *Eucobresia*, *Insulivitrina* and *Phenacolimax*, or for *Plutonia sensu* Alonso et al. (2000). The most resolved group (but with low support values) was a clade including species of *Oligolimax*, *Sardovitrina*, *Semilimacella* and *Vitrina* supported by exclusive disposition of the penial retractor above the right ommatophore retractor. These results indicate that a different approach to vitrinid phylogeny and systematics is needed because morphological characters alone do not generate a realistic picture. In the meantime, we ranked the 18 genus-group taxa as distinct genera, although we are perfectly aware that some may be paraphyletic or polyphyletic.

Finally we concisely surveyed all vitrinid genera, listing them in three distinct groups according to stimulator structure: vitrinids with vaginal stimulator (glandula amatoria); vitrinids with diverticular stimulator (atrial/atrial-vaginal/vaginal or penial diverticular stimulator); vitrinids without vaginal or diverticular stimulator. This approach enables easy comparison of taxa with similar distal genital structure and is not intended to have any systematic or phylogenetic value. A short diagnosis of each taxon is given together with re-description of the type species and remarks.

Key words: Vitrinidae, phylogeny, systematics, character evolution, description of new taxa.

INTRODUCTION

In recent years, the vitrinids have received much attention (Groh & Hemmen, 1986; Schileyko, 1986, 1991; Alonso et al., 1987, 2000; Ibáñez et al., 1987, 2001; Morales et al., 1988; Valido et al., 1990, 1993, 2000; Hausdorf, 1995, 2002; Wiktor & Backeljau, 1995; Neubert, 1998; Manganelli & Giusti, 2004, 2005; Nardi et al., 2007). Most of these contributions, however, have been concerned with taxonomy (revision of taxa and description of new genera/

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*Corresponding author: giustif@unisi.it

subgenera and species); only Hausdorf (1995, 2002) and Alonso et al. (2000) investigated their internal phylogeny.

Hausdorf (1995) used a cladistic approach to explore the relationships of the new species *Gallandia olympica* with *Gallandia annularis* and *Trochovitrina lederi,* using *Insulivitrina* as outgroup. He found that these three taxa constitute a monophyletic group (*G. annularis* (*G. olympica*, *T. lederi*)) and consequently regarded *Trochovitrina* as a junior synonym of *Gallandia* (the latter in turn is a junior synonym of *Oligolimax*; Manganelli & Giusti, 2004).

Alonso et al. (2000) analyzed the relationships of the "plutoniine" vitrinids (*Arabivitrina*, *Canarivitrina*, *Gallandia*, *Guerrina*, *Insulivitrina*, *Madeirovitrina*, *Phenacolimax* and *Plutonia*) using *Semilimax* as outgroup. They found eight equally parsimonious trees with a largely unresolved strict consensus tree (*Semilimax* (*Phenacolimax* (*Arabivitrina*, *Canarivitrina*, *Gallandia*, *Guerrina*, *Insulivitrina*, *Madeirovitrina*, *Plutonia*))). They adopted a largely unresolved tree (*Semilimax* (*Phenacolimax* (*Arabivitrina*, *Gallandia* (*Canarivitrina*, *Guerrina*, *Insulivitrina*, *Madeirovitrina*, *Plutonia*)))) as the preferred phylogenetic hypothesis and accordingly regarded all Macaronesian taxa (*Canarivitrina*, *Guerrina*, *Insulivitrina*, *Madeirovitrina* and *Plutonia*) as subgenera of *Plutonia*.

Hausdorf (2002) analyzed the phylogeny of all the genera assigned to the family, using *Troglaegopis* Riedel & Radja, 1983 (Zonitidae, Zonitoidea) and *Cryptozona* Mörch, 1872 (Ariophantidae, Helicarionoidea) as outgroups. On the basis of a character matrix of 12 taxa and 17 characters, he found two equally parsimonious trees which gave the following strict consensus tree: (*Semilimax*, *Vitrinobrachium* ((*Semilimacella*, *Vitrina*) (*Eucobresia* (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*)))))). Unfortunately, Hausdorf's phylogenetic study has some shortcomings due to the fact that he used supraspecific taxa whose monophyly was implicitly assumed, scored characters partly from the literature and did not calculate any statistical support to clades.

We therefore conducted a new phylogenetic study on the basis of the morphological characters of all the valid genus-group taxa using an exemplar approach. The aim was: (1) to investigate relationships between genus-group taxa, (2) to test their monophyly, and (3) to revise current taxonomy.

MATERIAL AND METHODS

Taxonomic Sample

Hausdorf (2002) and Schileyko (2003) proposed the most recent supraspecific classification of the family Vitrinidae (Table 1). Although the number of genus-group taxa is almost equivalent (14 according to Hausdorf vs. 15 according to Schileyko), the arrangement is quite different. First of all, Schileyko continued to use *Oligolimax* in the sense of Forcart (1965) for *Semilimacella* and assigned species included in *Oligolimax* by all other authors to *Phenacolimax*. As proposed by Alonso et al. (2000), Hausdorf accepted that the Macaronesian vitrinids belong to a polytypic genus (*Plutonia*, with five subgenera: *Canarivitrina*, *Guerrina*, *Insulivitrina*, *Madeirovitrina* and *Plutonia* s.s.), whereas Schileyko assigned them to four distinct genera (*Guerrina*, *Insulivitrina*, *Madeirovitrina* and *Plutonia*), one of which was polytypic (*Plutonia*, with two subgenera: *Canarivitrina* and *Plutonia* s.s.). Finally Hausdorf regarded *Hessemilimax* (a subgenus of *Semilimax*, according to Schileyko) and *Trochovitrina* (a distinct genus, according to Schileyko) as junior synonyms of *Semilimax* and *Oligolimax*, respectively.

Our analysis considered all the genus-group taxa listed as valid by Hausdorf and Schileyko plus *Sardovitrina* recently described by us (Manganelli & Giusti, 2004) and the new genus *Azorivitrina* (Table 1), making a total of 18 taxa of the genus group. In order to avoid *a priori* assumption of monophyly of supraspecific taxa, an exemplar approach with individual species as terminals was used (Prendini, 2000). Consequently at least two species, one of which was the type species, were examined, when possible, for each polytypic genus-group taxon.

Morphological Study

For each species considered, detailed anatomical re-examination was performed on the available material. The bodies were isolated removing them from the shells and dissected under the light microscope (Wild M5A) using very fine pointed watchmaker's tweezers. Anatomical details were drawn using a Wild camera lucida.

The material examined is listed as follows, when possible: locality, municipality and province names in parenthesis, UTM reference,

TABLE 1. Classification of the vitrinids according to Hausdorf (2002) and Schileyko (2003) and taxa of the genus group considered in the present survey.

Hausdorf (2002)	Schileyko (2003)	Taxa considered in the present survey
Vitrinidae Fitzinger, 1833 *Arabivitrina* Thiele, 1931 *Calidivitrina* Pilsbry, 1919 *Eucobresia* Baker, 1929 *Oligolimax* Fischer in: Paulucci, 1878 *Phenacolimax* Stabile, 1859 *Plutonia* Stabile, 1864 *Canarivitrina* Valido & Alonso in: Alonso et al., 2000 *Guerrina* Odhner, 1954 *Insulivitrina* Hesse, 1923 *Madeirovitrina* Groh & Hemmen, 1986 *Plutonia* s.s. Stabile, 1864 *Semilimacella* Soós, 1917 *Semilimax* Stabile, 1859 *Vitrina* Draparnaud, 1801 *Vitrinobrachium* Künkel, 1929	**Vitrinidae Fitzinger, 1833** **Plutoniainae Cockerell, 1893** *Arabivitrina* Thiele, 1931 *Insulivitrina* Hesse, 1923 *Phenacolimax* Stabile, 1859 *Trochovitrina* Boettger, 1880 *Plutonia* Stabile, 1864 *Canarivitrina* Valido & Alonso in: Alonso et al., 2000 *Plutonia* s.s. Stabile, 1864 *Guerrina* Odhner, 1954 *Madeirovitrina* Groh & Hemmen, 1986 **Semilimacinae Schileyko, 1986** **Oligolimacini Schileyko, 2003** *Oligolimax* Fischer in: Paulucci, 1878 **Semilimacini Schileyko, 1986** *Semilimax* Stabile, 1859 *Semilimax* s.s. Stabile, 1859 *Hessemilimax* Schileyko, 1986 *Vitrinobrachium* Künkel, 1929 *Eucobresia* Baker, 1929 **Vitrininae Fitzinger, 1833** *Calidivitrina* Pilsbry, 1919 *Vitrina* Draparnaud, 1801	**Vitrinidae Fitzinger, 1833** *Arabivitrina* Thiele, 1931 *Azorivitrina* n. gen. *Calidivitrina* Pilsbry, 1919 *Canarivitrina* Valido & Alonso in: Alonso et al., 2000 *Eucobresia* Baker, 1929 *Guerrina* Odhner, 1954 *Hessemilimax* Schileyko, 1986 *Insulivitrina* Hesse, 1923 *Madeirovitrina* Groh & Hemmen, 1986 *Oligolimax* Fischer in: Paulucci, 1878 *Phenacolimax* Stabile, 1859 *Plutonia* Stabile, 1864 *Sardovitrina* Manganelli & Giusti, 2005 *Semilimax* Stabile, 1859 *Semilimacella* Soós, 1917 *Trochovitrina* Boettger, 1880 *Vitrina* Draparnaud, 1801 *Vitrinobrachium* Künkel, 1929

collector(s), date, number of specimens and collection in which material is kept in parenthesis. Key to acronyms used in material examined: FGC, F. Giusti collection (Dipartimento di Scienze Ambientali, Università di Siena, Siena, Italy); IRSNB, Institut Royal des Sciences Naturelles de Belgique (Brussels, Belgium); MNHN, Muséum National d'Histoire Naturelle (Paris, France); NHMW, Naturhistorisches Museum Wien (Vienna, Austria); SMF, Senckenberg-Museum (Frankfurt am Main, Germany); ZMMU, Zoological Museum of Moscow University (Moscow, Russia).

Key to acronyms used in figures: A, atrium; AAVS, apical portion of atrial-vaginal stimulator; AG, albumen gland; AP, accessory pilaster; AS, atrial stimulator; ASC, atrial semicircular crest; AVS, apical portion of vaginal stimulator; BAVS, basal portion of atrial-vaginal stimulator; BC, bursa copulatrix; BVS, basal portion of vaginal stimulator; BW, body wall; CS, coagulated secretion; CSS, conical structure of stimulator; DBC, duct of bursa copulatrix; DV, distal vagina; EGL, external glandular layer; FHD, first hermaphrodite duct; FO, free oviduct; G, gonad; GA, glandula amatoria; GN, genital nerve; GR, glandular roll; IGL, internal glandular layer; L, lumen; LGR, lamellate portion of glandular roll; MP, main pilaster; MS, muscular strips; OSD, ovispermiduct; PAVS, papilla of atrial-vaginal

TABLE 2. Character matrix used for the phylogenetic analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Deroceras laeve (Müller, 1774)	2	0	0	1	0	-	0	1	0	0	1	-	1	1	-	-	2	0	0	0	0	-	-	-	1
Euconulus fulvus (Müller, 1774)	0	1	0	0	2	-	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Oxychilus cellarius (Müller, 1774)	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	-	-	-	0
Tandonia budapestensis (Hazay, 1881)	2	1	0	0	0	-	0	0	0	0	1	-	0	1	-	-	0	0	0	0	0	-	-	-	1
Troglaegopis mosorensis (Kuščer, 1933)	0	0	1	0	2	-	0	1	0	0	1	-	0	1	-	-	1	0	0	0	0	1	1	1	0
Vitrea crystallina (Müller, 1774)	0	0	0	0	0	-	0	1	0	1	0	0	0	1	-	-	1	0	0	0	0	-	-	-	1
Arabivitrina arabica (Thiele, 1910)	1	1	0	0	1	0	1	1	0	0	0	0	2	1	-	-	1	1	0	0	0	-	-	-	2
Arabivitrina jansseni Neubert, 1998	1	1	0	0	1	0	1	1	0	0	0	0	2	1	-	-	1	1	0	0	0	-	-	-	2
Azorivitrina brevispira (Morelet, 1860)	1	1	0	0	0	-	0	1	0	0	0	1	2	1	-	-	2	0	0	0	1	-	-	-	2
Azorivitrina finitima (Morelet, 1860)	1	1	0	0	1	0	1	1	0	0	0	1	2	0	?	2	1	0	0	0	1	-	-	-	2
Azorivitrina laxata (Morelet, 1860)	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	1	1	0	0	1	-	-	-	2
Canarivitrina dianae (Valido & Alonso in: Alonso et al., 2000)	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	3	0	1	0	0	-	-	-	2
Canarivitrina taburientensis (Groh & Valido in: Alonso et al., 2000)	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	3	0	1	0	0	-	-	-	1
Guerrina christinae Groh in: Valido et al., 1993	0	1	0	0	1	1	1	1	0	0	0	1	2	1	-	-	2	1	0	0	0	-	-	-	1
Guerrina cuticula (Shuttleworth, 1852)	0	1	0	0	1	1	1	1	0	0	0	1	2	1	-	-	2	1	0	0	0	-	-	-	2
Insulivitrina eceroensis Alonso & Ibáñez, 1987	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	2	0	0	0	0	-	-	-	1
Insulivitrina lamarckii (Férussac, 1821)	1	1	0	0	1	0	1	1	0	0	0	1	2	0	1	2	1	0	0	0	0	-	-	-	2
Madeirovitrina marcida (Gould, 1847)	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	2	1	0	1	0	-	-	-	2
Madeirovitrina nitida (Gould, 1847)	1	1	0	0	1	0	1	1	0	0	0	1	2	1	-	-	2	1	0	1	0	-	-	-	2

(continues)

(continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Oligolimax annularis (Studer, 1820)	0	1	0	0	1	1	1	1	0	0	1	-	2	1	-	-	1	0	0	0	0	-	-	-	1
Oligolimax musignani (Pirajno, 1842)	0	1	0	0	1	1	1	1	0	0	1	-	2	1	-	-	1	0	0	0	0	-	-	-	1
Phenacolimax blanci (Pollonera, 1884)	1	1	0	0	1	1	1	1	0	0	0	0	2	0	0	2	1	0	0	0	0	-	-	-	2
Phenacolimax major (Férussac, 1807)	1	1	0	0	1	1	1	1	0	0	0	0	2	0	1	2	1	0	0	0	0	-	-	-	0
Plutonia atlantica (Morelet, 1860)	2	1	0	0	1	0	1	1	1	0	-	-	2	1	-	-	1	0	0	0	0	-	-	-	2
Sardovitrina polloneriana ([Arbanasich], 1897)	1	1	0	0	1	1	1	1	0	0	1	-	2	1	-	-	1	0	0	0	0	-	-	-	2
Trochovitrina lederi (Boettger, 1878)	1	?	-	0	1	0	0	1	0	0	?	?	2	1	-	-	2	0	0	0	0	-	-	-	1
Hessemilimax kotulae (Westerlund, 1883)	1	1	0	0	2	-	0	1	0	0	0	1	2	0	0	2	2	0	0	0	0	2	1	1	2
Semilimacella bonelli (Targioni Tozzetti, 1873)	1	1	1	0	2	-	0	1	0	0	1	-	2	0	0	2	1	0	0	0	0	1	1	3	1
Semilimacella cephalonica (Rähle, 1980)	1	?	0	0	2	-	0	1	0	0	1	-	2	0	0	2	2	0	0	0	0	1	1	1	1
Semilimax carinthiacus (Westerlund, 1886)	1	1	0	0	2	-	0	1	0	0	0	1	2	0	0	2	1	0	0	0	0	2	1	2	2
Semilimax pyrenaicus (Férussac, 1821)	1	1	0	0	2	-	0	1	2	-	-	-	2	0	0	2	1	0	0	0	0	2	1	1	2
Semilimax semilimax (Férussac, 1802)	1	1	0	0	2	-	0	1	2	-	-	-	2	0	0	2	1	0	0	0	0	2	1	2	2
Vitrinobrachium baccettii Giusti & Mazzini, 1971	1	1	0	1	2	-	0	1	0	1	0	0	2	0	1	2	1	0	0	0	0	1	0	4	2
Vitrinobrachium breve (Férussac, 1821)	1	1	0	1	2	-	0	1	0	1	0	0	2	0	1	2	1	0	0	0	0	1	0	4	2
Calidivitrina cf. *oleosa* (Martens, 1895)	1	?	0	0	0	-	3	1	0	0	?	?	2	1	-	-	2	1	0	0	0	-	-	-	2
Eucobresia diaphana (Draparnaud, 1805)	1	1	0	0	0	-	3	1	0	0	0	0	2	0	1	2	1	0	0	0	0	-	-	-	1
Eucobresia glacialis (Forbes, 1837)	1	1	0	0	1	1	1	1	0	0	0	0	2	0	1	2	1	0	0	0	0	-	-	-	1
Eucobresia nivalis (Dumont & Mortillet, 1852)	1	1	0	0	0	-	2	1	0	0	0	0	2	0	1	2	1	1	0	0	0	-	-	-	2
Vitrina pellucida (Müller, 1774)	1	1	1	0	0	-	0	1	0	0	1	-	2	0	1	2	1	0	0	0	0	-	-	-	2

stimulator; PC, penial complex; PG, penial gland; PGA, papilla of glandula amatoria; PLC, penial laminar crest; PLS, papilla-like structure; PN, penial nerve; POS, prostatic portion of ovispermiduct; PR, penial retractor muscle; PS, penial sheath; PST, penial stimulator; SPS, pedunculate sucker of stimulator; T, talon; TLS, tongue-like structure; UOS, uterine portion of ovispermiduct; V, vagina; VD, vas deferens; VDO, vas deferens opening; VLC, vaginal laminar crest; VLP, vaginal longitudinal pilaster/pleat; VP, vaginal papilla; VW, vaginal walls.

In the anatomical description the use of directional (proximal and distal; basal and apical) and descriptive terms (short vs. long, slender vs. wide, etc.) was applied as follows. In the case of the ducts of the main axis of the genitalia (e.g., free oviduct, vagina, vas deferens, penial complex, etc.), proximal denotes the part which is closer to the gonad and distal the part which is closer to the gonopore. In the case of the blind structures radiating from the main axis of the genitalia (bursa copulatix complex, penial/vaginal/atrial appendix, etc.), basal or initial denotes the part closer to, and apical or terminal the part further from the main axis of the genitalia; in the case of the penial sheath, basal or initial denotes the part closer to, and apical or terminal the part further from where the sheath joins the penial complex. The descriptive terms (e.g., short vs. long, slender vs. wide, etc.) refer to comparisons of the same tract of the genitalia in different taxa. Unfortunately, only extreme cases are easy to evaluate; intermediate cases are very difficult to evaluate objectively due to large variability between taxa. Application of these simple categories is far from banal. Apparently simple structures (penial complex or even bursa copulatrix, duct of bursa copulatix, etc.) may be so different in different taxa as to elude any attempt at objective comparison. Besides variability between taxa, sometimes variations may be due to different sexual maturation of reproductive organs, fixation, and in the case of drawings in the literature, different fidelity of illustration. Only examination of a considerable number of specimens by the same researcher would provide real insights, but this was not possible due to unavailability of material.

Characters

Twenty-five characters (Table 2) were scored during examination of 33 species belonging to the in-group. Other potential characters were considered (e.g., penial complex length, distal vagina length, bursa copulatrix shape), but in the end they were disregarded due to the difficulty of coding and evaluating (see morphological study). Other characters recently used for phylogenetic analysis of the vitrinids, or of limacoideans, are sole, spermatophore, sexual behaviour and radula (see: Muratov, 1999; Hausdorf, 2002). Although they are interesting, we excluded them because data was only available for a handful of species.

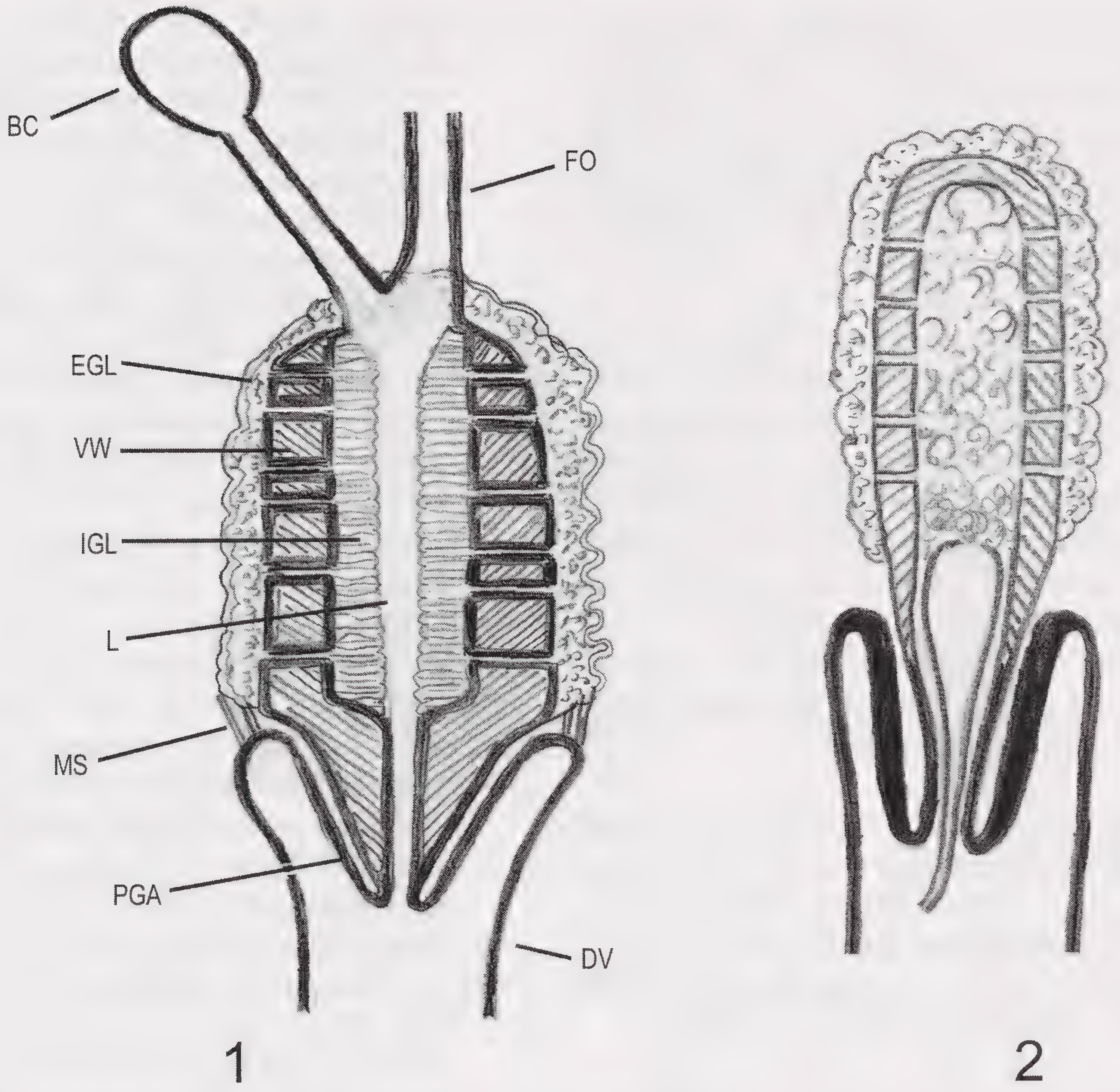
1. *Habitus*: 0 = snail-like (animal can completely withdraw into shell); 1 = semislug-like (the animal cannot completely withdraw into shell); 2 = slug-like. State 2 is unique to *Plutonia atlantica*. This character was also used by Alonso et al. (2000: tables 2, 3, character 1 "Shell"), but not by Hausdorf (2002).

2. *Penial innervation*: 0 = from cerebral ganglion; 1 = from pedal ganglion. State 0 is found in *Deroceras laeve* (Hausdorf, 1998: table 3, character 14, as Agriolimacidae), *Oxychilus cellarius*, *Vitrea crystallina* (Hausdorf, 1998: table 3, character 14, as Vitreidae) and *Troglaegopis mosorensis*; state 1 is found in *Euconulus fulvus*, *Tandonia budapestensis* and all the vitrinids examined.

3. *Bursa copulatrix duct*: 0 = initially not or only slightly flared; 1 = initially much flared. State 1 is only found in *Troglaegopis mosorensis*, *Semilimacella bonelli* and *Vitrina pellucida*. This character is not applicable to *Trochovitrina lederi* which apparently lacks bursa copulatrix.

4. *Vagina*: 0 = present; 1 = absent (vagina is absent when duct of bursa copulatrix is attached to penial complex). State 1 is only found in *Deroceras laeve* and species of *Vitrinobrachium.*

5. *Stimulator structures* (Figs. 1, 2): 0 = absent; 1 = glandula amatoria; 2 = diverticular stimulator. For other characters scored on diverticular stimulator, see nos. 22–24. Stimulator characters were also used by Alonso et al. (2000: tables 2, 3, character 3 "Location of the glandula amatoria – stimulator organ": 0 = distinct from vagina; 1 = fused to the proximal part of vagina) and Hausdorf (2002: table 1, character 1 "Stimulator": 0 = present; 1 = missing; character 2 "Stimulator": 0 = inserts laterally



FIGS. 1, 2. Vitrinid stimulator structures. FIG. 1: Glandula amatoria; FIG. 2: Diverticular stimulator (based on that of *Semilimax semilimax*).

at atrium or vagina; 1 = completely fused with vagina). There is general agreement that the various types of stimulator of the vitrinids are homologous (Schileyko, 1986; Hausdorf, 1998, 2002), but different assumptions have been made about their evolution. Hausdorf (1998, 2002) believed that the diverticular stimulator was primitive and that the vaginal stimulator (glandula amatoria) originated by fusion of it with the vagina. On the contrary Schileyko (1986) considered the glandula amatoria the primitive form of stimulator, directly derived from a sort of vaginal gland; in differentiating from the vagina, the glandula amatoria allegedly gave rise to the atrial/penial stimulator. We accepted the hypothesis that the vitrinid stimulators are homologous, but avoided any polarity assumption.

6. *External glandular covering of glandula amatoria*: 0 = uniform and complete or almost complete; 1 = reduced. This character is not applicable to taxa lacking glandula amatoria (see character 5). A continuous complete (or almost complete) external glandular covering of glandula amatoria is found in species of *Arabivitrina*, *Canarivitrina*, *Insulivitrina*, *Madeirovitrina*, *Plutonia* and *Trochovitrina*, whereas a reduced, incomplete external glandular covering is present in species of *Eucobresia*, *Guerrina*, *Phenacolimax*, *Oligolimax* and *Sardovitrina*. Simple glandular covering, not associated with a glandula amatoria, is also present around proximal vagina of *Oxychilus cellarius*, *Semilimacella bonelli* and *Vitrina pellucida*.

Hausdorf (2002) regarded the external glandular covering of glandula amatoria and that of appendicular stimulators as homologous and scored on it three characters, according to presence vs. absence (character 3: 0 = present; 1 = absent), length (character 4: 0 = distinctly longer than wide; 1 = about as long as or shorter than wide) and extension around proximal portion of vaginal stimulator or apical portion of diverticular stimulator (character 5: 0 = uniform sheath around proximal stimulator portion; 1 = divided into lobes). However, it is impossible to compare these structures in relation to the extent of their covering (one is a cylinder and the other a cup).

7. *Vaginal papilla*: 0 = absent; 1 = present; 2 = reduced; 3 = very reduced. A conical vaginal papilla is found in all vitrinids with glandula amatoria apparently except *Trochovitrina lederi*. A small proximal vaginal papilla is present in *Eucobresia nivalis* and a pleated proximal annular pad in *Calidivitrina* cf. *oleosa* and *Eucobresia diaphana*. The vaginal pad may actually be an extreme reduction of the glandula amatoria of a *Phenacolimax*-like ancestor.

8. *Epiphallus*: 0 = present; 1 = absent. State 0 subsists in *Euconulus fulvus*, *Oxychilus cellarius* and *Tandonia budapestensis*; state 1 in *Deroceras laeve*, *Troglaegopis mosorensis*, *Vitrea crystallina* (Barker, 1999: 117) and all the vitrinids examined.

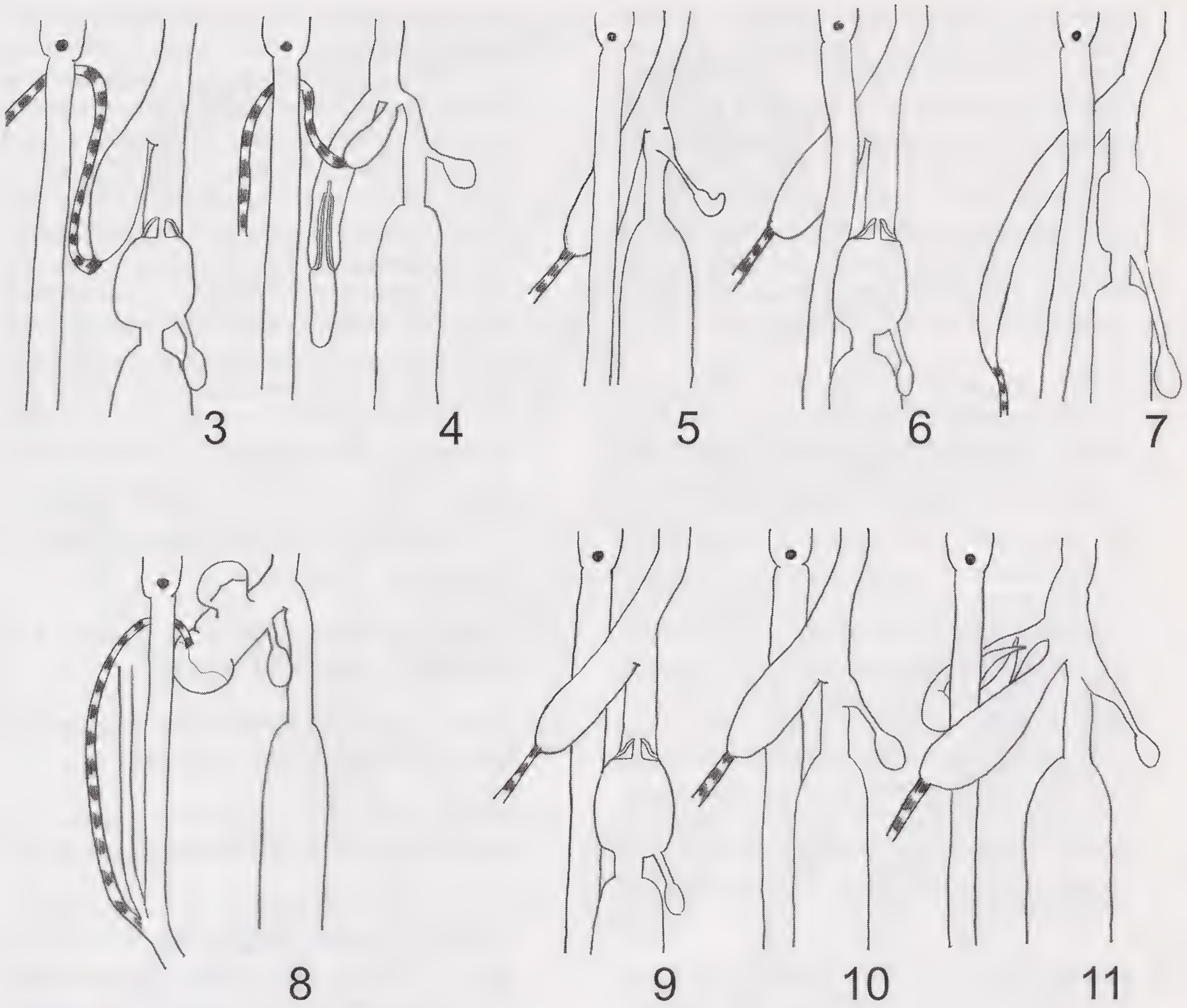
9. *Penial retractor*: 0 = present; 1 = subdivided into three branches, none of which reaches penial complex (the first reaches bursa copulatrix and its duct, second free oviduct and third vas deferens); 2 = absent. State 1 is only found in *Plutonia atlantica* and state 2 in two species of *Semilimax* (*S. semilimax* and *S. pyrenaicus*).

10. *Origin of penial retractor*: 0 = penial retractor from diaphragm; 1 = penial retractor from columellar muscle. State 1 is found in *Vitrea crystallina* (Barker, 1999: 117) and *Vitrinobrachium* species. This character is not applicable to taxa without penial retractor (see character 9) and was codified by Hausdorf (2002: table 1, character 14 "Insertion site of the penial retractor muscle") in the same way.

11. *Right ommatophore retractor and penial retractor in adults* (Figs. 3–11): 0 = ROR above PR/PC; 1 = ROR below PR/PC. As in the case of characters 9–10, this character is not applicable to taxa with modified or no PR (see character 9). When PR is short, PC is displaced left and located below or above ROR. In the first case (ROR above PC: adults of *Arabivitrina*, *Eucobresia* and *Phenacolimax*), PR does not have an S-like course around the optic nerve and ROR passes between penial complex and vagina. In the second case (ROR below PC: species of *Oligolimax*, *Sardovitrina*, *Semilimacella* and *Vitrina*), ROR runs free of both optic nerve and distal genitalia. When PR is long and has an S-like course around the optic nerve (juveniles of *Arabivitrina*, *Eucobresia* and *Phenacolimax* and juveniles and adults of all Macaronesian vitrinids, *Semilimax* and *Vitrinobrachium*), ROR passes far left of PC and above PR (see character 12).

This character was codified by Alonso et al. (2000: tables 2, 3, character 7 "Arrangement of the penial retractor muscle") in three states: 0 = PR free of right optic nerve [= ROR below PR]; 1 = PR passing around right optic nerve [= ROR above PR]; 2 = PR absent (*Plutonia*) and by Hausdorf (2002: table 1, character 13 "Right ommatophore retractor muscle") in two states: 0 = ROR runs left of or below PR (ROR can be moved to left without affecting PR); 1 = ROR runs right of or above PR (ROR cannot be moved to left without affecting PR). In both cases, their state 0 corresponds to our state 1 and vice versa (our codification depends on the fact that we listed *Oxychilus* (0) as first outgroup). Alonso et al. (2000) assigned state 0 to *Arabivitrina* and *Semilimax* (PR free of right optic nerve) but this is a mistake.

12. *Right ommatophore retractor and penial retractor in adults when ROR is above PR* (Figs. 3–11): 0 = PR short, PC shifted left and ROR between distal genitalia (species of *Arabivitrina*, *Eucobresia*, *Phenacolimax*); 1 = PR long and with S-like course around optic nerve, PC located right and ROR not between distal genitalia. The position of ROR with respect to the distal genitalia depends on the length of PR which may change between juveniles and adults of some genera (see *Arabivitrina* and *Phenacolimax*). This character was also considered by Hausdorf (2002: table 1, character 12 "Right ommatophore retractor muscle") and codified in this way: 0 = ROR does not pass between penis and the female genitalia (ROR can be moved to left



FIGS. 3–11. Scheme of relationships between distal genitalia, penial retractor and right ommatophore retractor. FIG. 3: *Canarivitrina*, *Guerrina*, *Insulivitrina* and *Madeirovitrina* and juveniles of *Arabivitrina* and *Phenacolimax*; FIG. 4: *Semilimax*; FIG. 5: *Eucobresia*; FIG. 6: *Calidivitrina* and adults of *Arabivitrina* and *Phenacolimax*; FIG. 7: *Oxychilus*; FIG. 8: *Vitrinobrachium*; FIG. 9: *Oligolimax* and *Sardovitrina*; FIG. 10: *Vitrina*; FIG. 11: *Semilimacella.*

without affecting the penis); 1 = ROR passes between penis and the female genitalia (ROR cannot be moved to left without affecting penis). We do not agree with this codification because ROR position with respect to distal genitalia cannot be compared in vitrinids with ROR above the PR/PC and those with ROR below PR/PC (see character 11). In fact Hausdorf erroneously considered the situations of *Oligolimax*, *Semilimacella* and *Vitrina* (short PR, ROR below the PC) and those of *Plutonia* (s. l.) and *Vitrinobrachium* (long PR, ROR above the PC) to be homologous.

13. *Penial gland*: 0 = absent; 1 = only distal; 2 = complete or only proximal. State 0 subsists in *Euconulus fulvus*, *Oxychilus cellarius*, *Tandonia budapestensis*, *Troglaegopis mosorensis* and *Vitrea crystallina*; state 1 is found in *Deroceras laeve*; state 2 in all vitrinids.

14. *Penial sheath*: 0 = present; 1 = absent. The penial sheath is absent in four species of the outgroup (*Deroceras laeve*, *Tandonia budapestensis*, *Troglaegopis mosorensis* and *Vitrea crystallina*), in species of *Calidivitrina*, *Arabivitrina*, *Oligolimax*, *Sardovitrina*, *Trochovitrina*

and all Macaronesian vitrinids except *Azorivitrina finitima*, *Insulivitrina blauneri* and *Insulivitrina lamarckii*. This character was also considered by Alonso et al. (2000: tables 2–3, character 6 “Penial sheath”) and Hausdorf (2002: table 1, character 8 “Penial tunica”). However Hausdorf (2002) erroneously assigned state 0 (penial sheath present) to *Troglaegopis*, possibly due to the fact that Zhiltsov & Selivanova (2000) called the penial wall “penial sheath”.

15. *Origin of penial sheath*: 0 = PS joins penial complex distally; 1 = PS joins penial complex medially. This character is not applicable to the taxa without penial sheath (see character 14). It was also used by Hausdorf (2002: table 1, character 9 “Penial tunica”) though differently codified: PS covering especially the distal penial complex (0: *Semilimacella*, *Semilimax*, *Vitrina* and *Vitrinobrachium*); PS covering the proximal penial complex (1: *Eucobresia* and *Phenacolimax*). Actually, the most important feature is not what the PS covers but the point where it joins the penial complex. In fact, PS is often wrinkled up, giving an erroneous impression of its real length (see for example, *Vitrina pellucida*: Figs. 103, 104).

16. *Penial sheath and vas deferens*: 0 = not traversed by vas deferens; 1 = apically traversed; 2 = basally traversed by vas deferens. State 0 is unique to *Euconulus fulvus.* State 1 is unique to *Oxychilus cellarius.* This character is not applicable to taxa lacking the penial sheath (see characters 14 and 15).

17. *Vas deferens entering penis*: 0 = vas deferens entering penial complex via epiphallus; 1 = vas deferens entering penial complex apically; 2 = vas deferens entering penial complex proximally/medially; 3 = vas deferens entering penial complex distally (*Canarivitrina* species). This character was also used by Hausdorf (2002: table 1, character 15 “Insertion of the vas deferens at the penis”) but differently codified: terminally (0: *Semilimacella* and *Vitrina*); subterminally or laterally (1: all the others).

18. *Main pilaster, glandular roll*: 0 = without a medial lamellate portion; 1 = with a medial lamellate portion. This character was also used by Hausdorf (2002: table 1, character 10 “Main pilaster in penis”) and similarly codified: without a short lamellate section or distal section divided (0: all taxa except *Arabivitrina*, *Calidivitrina* and *Plutonia* in part); with a short lamellate section and an undivided distal section (1: *Arabivitrina*, *Calidivitrina* and *Plutonia* in part). State 1 is clearly present in *Arabivitrina*, *Calidivitrina*, *Guerrina*, *Madeirovitrina* and in two species of *Plutonia sensu* Mordan & Martins (2001) (*P. laxata* and *P. pelagica*). A glandular roll (or its derivative), more or less distally pleated, is also present in *Canarivitrina*, *Eucobresia*, *Insulivitrina*, *Oligolimax* and *Phenacolimax*, but it is not clear whether it corresponds to the lamellate glandular roll of the other taxa.

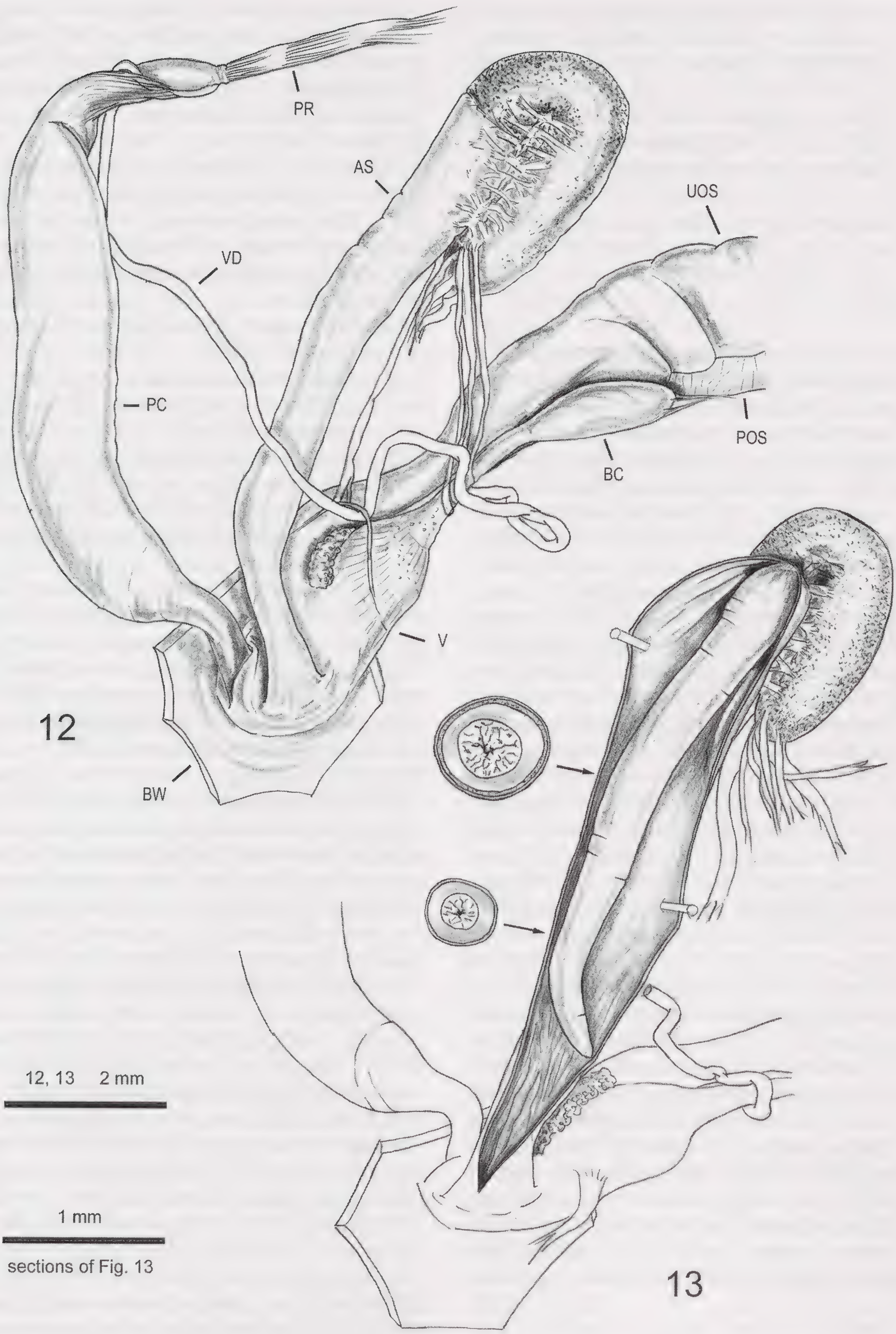
19. *Penial laminar crests*: 0 = absent; 1 = present (*Canarivitrina* species).

20. *Penial bridge-like pilaster*: 0 = absent; 1 = present (*Madeirovitrina* species).

21. *Penial pouch-like pilaster*: 0 = absent; 1 = present (Azorean “*Insulivitrina*” species).

22. *Diverticular stimulator*: 0 = finger-like without apical glandular portion; 1 = finger-like or club-like with apical glandular cap; 2 = finger-like or club-like with an apical pedunculated sac-like portion invaginated into basal portion. This character is not applicable to the taxa without stimulator structures (see character 5). Diverticular stimulators are present in *Euconulus fulvus*, *Troglaegopis mosorensis*, species of *Hessemilimax*, *Semilimax*, *Semilimacella* and *Vitrinobrachium* (Fig. 2). Contrary to what Hausdorf (2002) stated the external glandular covering of the apical portion of stimulator of *Semilimacella* is complete (it is perfectly equivalent to that of *Vitrinobrachium*).

23. *Diverticular stimulator location*: 0 = penial (penial stimulator); 1 = atrial or atrial-vaginal or vaginal (atrial/atrial-vaginal/vaginal stimulator). This character is not applicable to taxa without stimulator structures (see character 5). Hausdorf (2002) considered the stimulator of *Vitrinobrachium* to be atrial, whereas it is clearly penial.



FIGS. 12, 13. *Troglaegopis mosorensis* (Kuščer, 1933) from Špilja Vranjača, Kotlenica near Split (Croatia), A. Riedel leg. 11.5.1999. FIG. 12: Distal genitalia; FIG. 13: Internal structure and two transverse sections of atrial appendix.

24. *Diverticular stimulator internal structure*: 0 = absent; 1 = conical papilla; 2 = conical papilla with internal chitinous structure; 3 = two chitinous conical structures; 4 = fungiform papilla. This character is not applicable to the taxa without stimulator structures (see character 5).

25. *Genital atrium*: 0 = short; 1 = medium; 2 = long.

Outgroup

There is general agreement that the vitrinids are limacoideans, a group of higher pulmonates (Hausdorf, 1998; Wade et al., 2001, 2006). However, uncertainty still exists about their relationships (historical accounts: Forcart, 1956; Hausdorf, 1998, 2002; Muratov, 1999). According to a phylogeny of limacoideans based on morphological characters (Hausdorf, 1998), the vitrinids are limacoideans *sensu stricto*, being the sister group of boettgerillid, limacid and agriolimacid slugs. Recent phylogenies of a large series of stylommatophorans based on analysis of ribosomal (r) RNA gene cluster (Wade et al., 2001, 2006) confirmed that they are limacoideans, but their relationships are uncertain. Based on these phylogenies, we opted for a composite outgroup consisting of some west Palaearctic limacoidean genera examined by Wade et al. (2001, 2006) (*Euconulus*, *Deroceras, Oxychilus*, *Tandonia* and *Vitrea*) plus *Troglaegopis*. The latter (Figs. 12, 13) was included because it was used as outgroup by Hausdorf (2002) (actually Hausdorf used an outgroup of two taxa, but the second is the Indomalayan ariophantid *Cryptozona*).

Among the genera included in the outgroup, *Euconulus* and *Oxychilus* share a similarly structured penial sheath (namely a thin, short to long sac joined to a distal or medial constriction of the penial complex at one end and open at the other) with many vitrinids. Moreover, the penial sheath of the oxychiline zonitids and the vitrinids is uniquely traversed by vas deferens on one side (basally in the vitrinids, apically in oxychiline zonitids) and this makes the oxychiline zonitids the best candidate for a sister group of the vitrinids.

Phylogenetic Analysis

Several analyses were performed using different composite outgroups formed by six, four or two taxa: outgroup formed by *Euconulus*, *Deroceras, Oxychilus*, *Tandonia*, *Vitrea* and *Troglaegopis* (data partition I, DPI); outgroup formed by *Euconulus*, *Oxychilus*, *Vitrea* and *Troglaegopis* (data partition II, DPII); outgroup formed by *Euconulus* and *Oxychilus* (data partition III, DPIII).

Parsimony analyses were performed using PAUP* 4.0 (Swofford, 2003) (MP analyses) and TNT (Tree analysis using New Technology) software (Goloboff et al., 2003) (AP analyses).

Parsimony analyses using PAUP* were carried as follows:

(1) characters unordered, equal weight of character states;
(2) characters unordered, equal weight of characters (regardless of number of character states);
(3) characters unordered, equal weight of character states except for: characters 11, 12, 19, 20 and 21, each weighted 20 times (arbitrarily, see Discussion) for DPI, DPII and DPIII.

Heuristic searches under equal weight of all characters were performed with the following options: 1,000 random stepwise additions; TBR branch swapping; saving only optimal trees over all replicates; no more than 100 trees at each replicate. To evaluate clade support, nonparametric bootstrap resampling was used (1,000 replicates).

A traditional search was run in TNT, with these options: heuristic searches using multiple random addition sequences and/or branch swapping. Shortest trees were found by processing 2000 different addition sequences by the tree bisection-reconnection branch-swapping method (TBR), retaining 100 trees per replicate. The characters were analysed using equal weigth of characters and implied weights (Farris, 1988; Goloboff, 1993, 1995; Ponssa, 2008). The more homoplastic characters (the more homoplastic, the less fit) are lower weight characters. Analyses were conducted each with constant of concavity (*k*) set to a different integer value from 1 to 6, where 1 is weighted most severely against homoplasious characters. The support of the clades was calculated by symmetric resampling (1000 replicates, with 10 addition sequences, saving up to 10 trees each) expressed as absolute frequencies (Goloboff et al., 2003), absolute and relative Bremer support (Goloboff & Farris, 2001).

Parsimony analysis using TNT was run on DPII and DPIII with *Euconulus* as outgroup since the software only works with one-taxon outgroup.

Bayesian inference (BI) analysis was run on DPIII (since it was the dataset on which more results were found by the other analyses) using MrBayes (version 3.1, Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Mkv models for discrete morphological data were applied to our dataset (Lewis, 2001). Two analyses were run: the first assuming equal rates of change among characters; the second with four rate categories of the gamma distribution parameter, allowing different character change rates across characters. Bayes factors (following Kass & Raftery, 1995; Nylander et al., 2004) were used to compare these two models and find model fitting evolution of the data best. Differences in harmonic means of log likelihoods of the two models multiplied by two were calculated. One model was preferred over the other for values > 10 (Nylander et al., 2004; Wiens, 2005). The default value of four Markov chains was used (one cold and three heated) and each chain was started from a random tree. The "temperature" parameter was set at 0.2. Monte Carlo Markov Chain length was 2,000,000 generations and trees were sampled every 100 generations. To establish whether the Markov chains had reached a steady state, we plotted the −ln likelihood scores of sampled trees against generation time. Log-likelihood scores were plotted to determine the number of trees to be discarded (i.e., to determine the length of the burn-in period). Only trees sampled after this burn-in period were used to construct a 50% majority rule tree.

RESULTS AND DISCUSSION

Vitrinid Phylogeny

Phylogeny analysis generally had low resolution although AP was better than the other methods (AP found 11 supported clades, MP 11 and BI 8; Table 3). AP also recovered all supported clades found by MP except four (clades 6, 9, 12 and 16) and by BI except two (clades 5 and 13).

Using Paup*, MP only revealed slight differences between the three datasets (DPI, DPII, DPIII) and almost never between the three analyses (1, 2 and 3). The analyses which produced more results were those on DPII and DPIII (8 and 9 supported clades respectively; DPII (3): length L = 486; consistency index CI = 0.273; retention index RI = 0.345; rescaled consistency index RCI = 0.094; DPIII (3): length L = 88; consistency index CI = 0.282; retention index RI = 0.287; rescaled consistency index RCI = 0.081).

Using TNT, AP revealed some differences between the two datasets (DPII, DPIII) (Table 3). Moreover, since the analyses with implied weigth showed the same or more results than those with equal weight, only the results under implied weigth are showed (Figs. 14, 15). For DPII (7 supported clades; Table 3), AP found 27 most parsimonious trees (tree length = 100 steps, CI = 38, RI = 56 for K = 3; Fig. 14) showing more basal groups, even if with low support, and two clades where *Vitrea crystallina* is sister group of the two *Vitrinobrachium* species and *Troglaegopis mosorensis* is grouped with *Semilimacella bonelli* and *Vitrina pellucida*. For DPIII (8 supported clades; Table 3), AP found 49 most parsimonious trees (tree length = 103 steps, CI = 35, RI = 44 for K = 3; Fig. 15).

As for BI analyses, the total harmonic means were -349.62 (equal rates of change among characters) and -346.87 (four rate categories of the gamma distribution parameter), and the resulting difference in harmonic means of log likelihoods of the two models multiplied by two was 5.5. Thus the gamma shape parameter is not preferred on the model, which assumes equal rates of change among characters. BI analysis recovered most of the clades found by MP plus two (*Arabivitrina arabica*, *A. jansseni*; and *Semilimacella bonelli*, *Semilimacella cephalonica*; Table 3).

Phylogeny analysis of DPI and DPII did not reveal monophyly of vitrinids, as MP and AP included some taxa of the outgroup in the ingroup. MP and AP analysis on DPII only found a basal, variably supported clade consisting of all vitrinids plus *Troglaegopis mosorensis* and *Vitrea crystallina* (MP: 55–60%; AP: 62% symmetric resampling, 0.23/38 Bremer absolute and relative supports). Only when the outgroup was reduced to two taxa (removing *Deroceras laeve*, *Tandonia budapestensis*, *Troglaegopis mosorensis* and *Vitrea crystallina*, DPIII), did the vitrinids prove to be a monophyletic group (MP: 88%; AP: 89% symmetric resampling, 0.61/62 absolute and relative Bremer supports) (Figs. 14, 15).

Apart from the basal inclusive groups, MP found nine monophyletic groups, none strongly supported except one (*Vitrinobrachium baccettii* - *V. breve*). All MP-supported monophyletic groups except three included only two terminal taxa; the three included the *Azorivitrina* species

TABLE 3. List of selected clades (clades with ≥ 50% bootstrap support for MP; ≥ 50% symmetric resampling support for AP with implied weigth and/or ≥ 7 and ≥ 50% absolute and relative Bremer support for AP with implied weigth) and their synapomorphies. MP: parsimony analyses by PAUP, AP: parsimony analyses using implied weights by TNT, BI: Bayesian inference by MRBAYES (equal weight), 1–3: analyses 1–3 (if not specified, clade appears in all analyses). When a clade appears in more than one analysis, the synapomorphies are in bold if they support the clade in all the analyses. *: only relative Bremer support.

No	Clade	MP	MP synapomorphies	AP	AP synapomorphies	BI
1	Vitrinids + *Oxychilus cellarius*, *Troglaegopis mosorensis*, *Vitrea crystallina*			DPII	**no common synapomorphies**	
2	Vitrinids + *Oxychilus cellarius*			DPIII	**no common synapomorphies**	
3	Vitrinids + *Troglaegopis mosorensis*, *Vitrea crystallina*	DPII	**1(1)** **5(1)** **7(1)** **8(1)** **13(2)** **14(1)** **17(1)** **25(2)**	DPII	**8(1)** **17(1)**	
4	Vitrinids	DPIII	**1(1)** **5(1)** **7(1)** **8(1)** **12(1)** **13(2)** **14(1)** **17(1)** **25(2)**	DPIII	**1(1)** **8(1)** **13(2)** **17(1)**	DPIII
5	*Arabivitrina arabica*, *Arabivitrina jansseni*					DPIII
6	*Azorivitrina brevispira*, *Azorivitrina finitima*, *Azorivitrina laxata*	DPI (3), DPII (3), DPIII (3)	**12(1)** 14(1) **21(1)**			
7	*Canarivitrina dianae*, *Canarivitrina taburientensis*	DPI, DPII, DPIII	12(1) 14(1) **17(3)** **19(1)**	DPII, DPIII	**19(1)**	DPIII
8	*Guerrina christinae*, *Guerrina cuticula*	DPI, DPII, DPIII	**1(0)** **6(1)** 12(1) 14(1) **17(2)** **18(1)**	DPII, DPIII	**1(0)**	DPIII
9	*Hessemilimax kotulae*, *Semilimax carinthiacus*, *Semilimax pyrenaicus*, *Semilimax semilimax*	DPII (2)	**5(2)** **7(0)** **12(1)** **14(0)** **15(0)** **22(2)**			
10	*Madeirovitrina marcida*, *Madeirovitrina nitida*	DPI, DPII, DPIII	12(1) 14(1) **17(2)** **18(1)** **20(1)**	DPII, DPIII	**20(1)**	DPIII
11	*Oligolimax annularis*, *Oligolimax musignani*	DPIII	**1(0)** **6(1)** **11(1)** **14(1)** **25(1)**	DPII*	**1(0)**	DPIII
12	*Oligolimax annularis*, *Oligolimax musignani*, *Sardovitrina polloneriana*, *Semilimacella bonelli*, *Semilimacella cephalonica*, *Vitrina pellucida*	DPIII (3)	**11(1)**			
13	*Semilimacella bonelli*, *Semilimacella cephalonica*					DPIII
14	*Semilimacella bonelli*, *Semilimacella cephalonica*, *Vitrina pellucida*			DPIII	**5(2)** **7(0)**	
15	*Semilimacella bonelli*, *Vitrina pellucida*			DPII*	**3(1)**	
16	*Semilimax pyrenaicus*, *Semilimax semilimax*	DPI (2), DPII (2, 3), DPIII (2, 3)	5(2) 7(0) **9(2)** 15(0) 22(2)			
17	*Vitrinobrachium baccettii*, *Vitrinobrachium breve*	DPI, DPII, DPIII	**4(1)** **5(2)** **7(0)** **10(1)** 12(0) 14(0) 15(1) **23(0)** **24(4)**	DPII, DPIII	**4(1)** 5(2) 10(1) 25(2)	DPIII

(1), *Hessemilimax kotulae* with the species of *Semilimax* (2), and species of *Oligolimax*, *Sardovitrina*, *Semilimacella* and *Vitrina* (3), respectively.

AP analysis produced well supported monophyletic groups (Table 3). Although most of the monophyletic groups consisted of only two terminal taxa, some were more inclusive (Figs. 14, 15).

Like MP, BI found a basal largely unresolved group and a few monophyletic groups, all consisting of two terminal taxa.

Vitrinid Systematics

Phylogenetic analysis did not support monophyly of most genus-group taxa or the monophyletic groups found by Hausdorf (1995, 2002) and Alonso et al. (2000), or the subfamilies and tribes established by Schileyko (1986, 2003), or the family Vitrinidae if the outgroup was conceived as consisting of a large selection of taxa of the larger monophyletic group to which vitrinids belongs, the limacoideans.

Regarding the genera, we found good support for monophyly of *Canarivitrina*, *Guerrina*, *Oligolimax* and *Vitrinobrachium* and sufficient support for monophyly of *Azorivitrina*. Notably three of these genera (*Canarivitrina*, *Guerrina* and *Azorivitrina*) are represented by insular endemics characterized by exclusive synapomorphies of the penial complex. On the contrary, we did not find any support for monophyly of *Eucobresia*, *Insulivitrina* and *Phenacolimax*, or for *Plutonia sensu* Alonso et al. (2000).

The most resolved group found was a clade including species of *Oligolimax*, *Sardovitrina*, *Semilimacella* and *Vitrina*, but with low support (MP: 50%; AP (DPIII): 9% symmetric resamplig, 0.15/40 absolute and relative Bremer supports; Figs. 14, 15, Table 3). This clade was supported by exclusive disposition of the penial retractor above the right ommatophore retractor. A sister-group relationship between *Oligolimax* and *Sardovitrina* was already suggested by Manganelli & Giusti (2005) when describing the latter genus. This clade includes taxa with glandula amatoria (*Oligolimax* and *Sardovitrina*), diverticular stimulator (*Semilimacella*) or lacking stimulatory structures (*Vitrina*). Relationships between *Semilimacella* and *Vitrina* were found by Hausdorf (2002); however those between the two species of *Semilimacella* and *Vitrina pellucida* are controversial: we found more support for the group *Semilimacella bonelli* – *Vitrina pellucida* than for that consisting of the two *Semilimacella* species (Table 3).

Another inclusive monophyletic group was represented by *Calidivitrina* cf. *oleosa* and species of *Guerrina* and *Madeirovitrina*, but it had low support (AP (DPII): 13% symmetric resampling, 0.07/36 absolute and relative Bremer supports; AP (DPIII): 16% symmetric resampling, 0.04/29 absolute and relative Bremer supports) (Figs. 14, 15). The group is characterized by two non exclusive synapomorphies (17(2) and 18(1)). It may be biased by the fact that it was only possible to examine two specimens (one adult already dissected and one juvenile) of a single species of *Calidivitrina*.

Our study failed to produce a definitive generic classification for the vitrinids, however it showed that *Plutonia* cannot be accepted in the sense of Alonso et al. (2000) or Schileyko (2003). We therefore prefer to rank the eighteen genus-group taxa considered in our survey as distinct genera, awaiting further research. We are perfectly aware that many of them may be paraphyletic or polyphyletic, but there is no alternative for the present. In the remarks to each genus we discuss the main problems related with its systematic status and relationships. Vitrinid phylogeny and systematics require a different approach because morphological characters alone are not able to generate a realistic phylogeny and systematics.

SYSTEMATIC REVISIONS

This section includes a concise survey of all the vitrinid genera, preceded by a redescription of the family. The genera are listed alphabetically in three distinct groups according to stimulator structure: vitrinids with vaginal stimulator (glandula amatoria) (1); vitrinids with diverticular stimulator (atrial/atrial-vaginal/vaginal or penial diverticular stimulator) (2); vitrinids without vaginal or diverticular stimulator (3). This approach was used to enable easy comparisons between taxa with similar distal genitalia structures and is not intended to have any systematic or phylogenetic value. For each taxon a short diagnosis, a description of the type species and some remarks are given.

VITRINIDAE Fitzinger, 1833

Type Genus: *Vitrina* Draparnaud, 1801.

Diagnosis

Semislug-like pulmonates with thin, glossy, translucent, ear-like dorsal shells partially

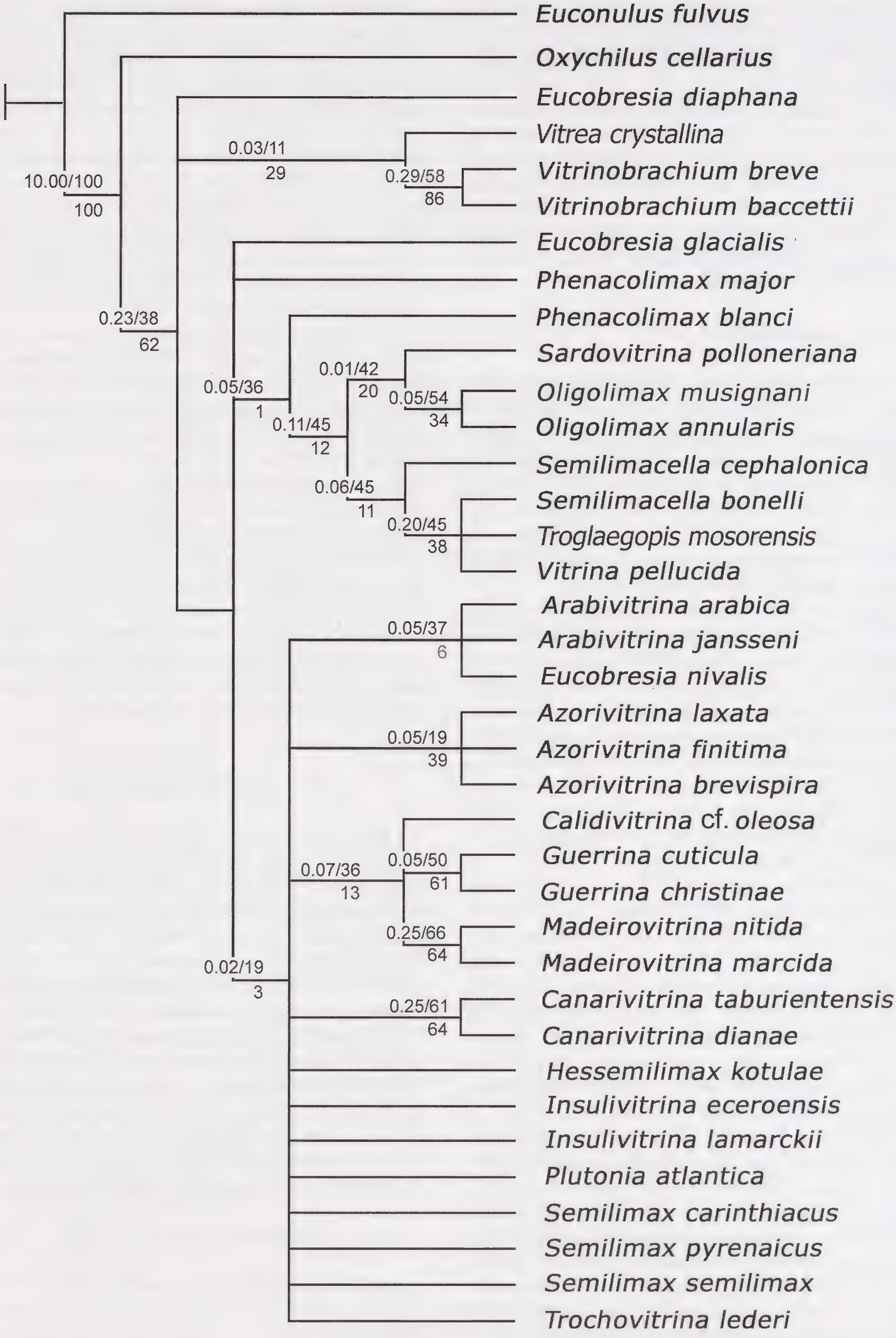


FIG. 14. Strict consensus of the 27 most parsimonious trees obtained under implied weights with concavity K = 3 on DPII (AP analysis). Values at nodes are absolute and relative Bremer support (above) and symmetric resampling (below).

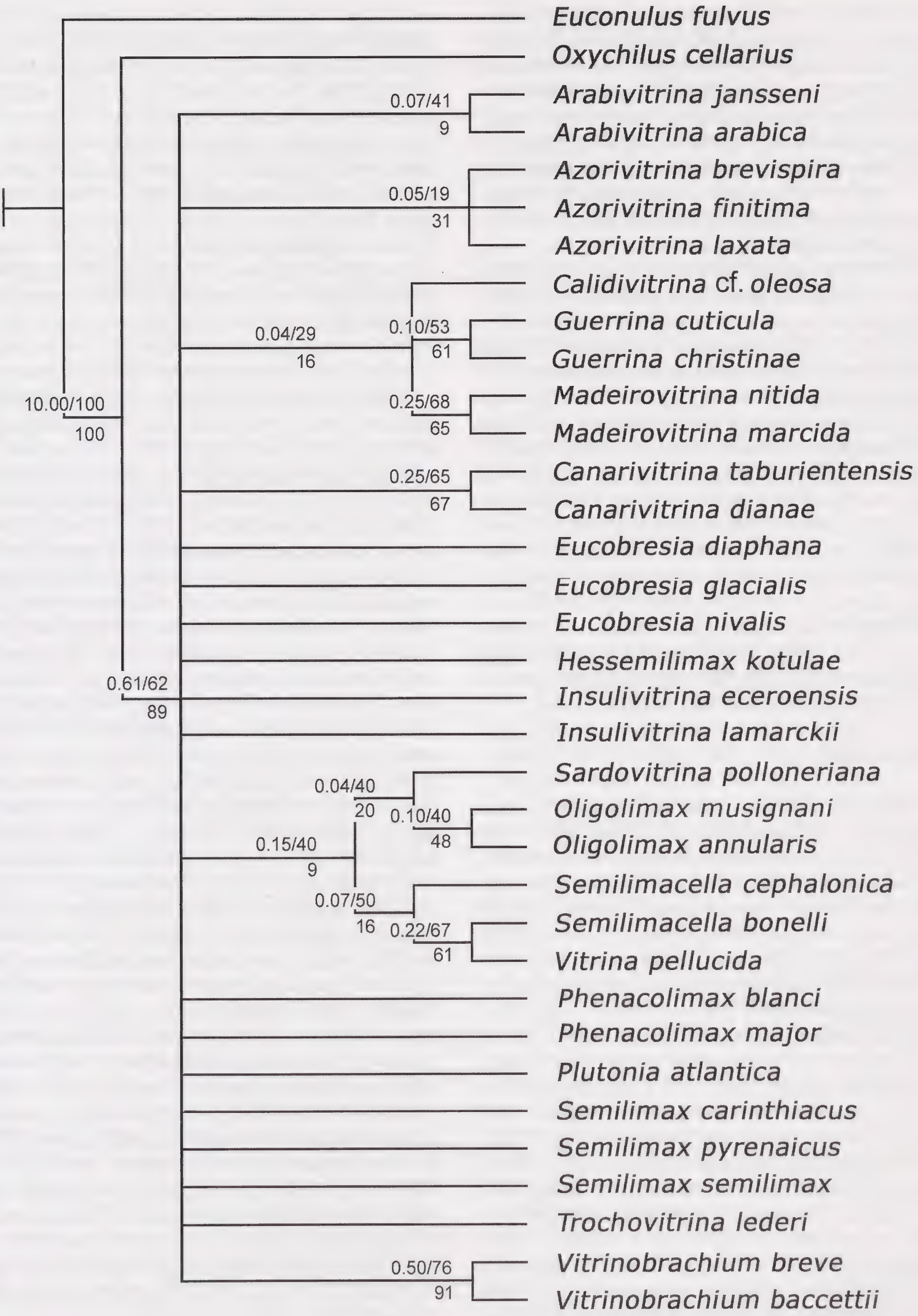


FIG. 15. Strict consensus of the 49 most parsimonious trees obtained under implied weights with concavity K = 3 on DPIII (AP analysis). Values at nodes are absolute and relative Bremer support (above) and symmetric resampling (below).

covered by large to very large anterior dorsal mantle shield and spatulate right shell lobe (though some derived taxa reverted to snail-like or acquired slug-like habitus); distal genitalia without epiphallus, with distinctive accessory stimulator structures consisting of vaginal glandula amatoria or distinct blind diverticulum associated with atrium or vagina or penial complex (but some derived taxa without accessory stimulator structures).

Within the geographical range of the family, no other similar semislugs exist. The western Palaearctic zonitids *Carpathica*, *Daudebardia* and *Libania* and the testacellid *Testacella* have a posterior shell and lack the mantle shield and a spatulate right shell lobe, while the parmacellids *Parmacella* and *Candaharia* have a dorsal shell consisting of a normal coiled protoconch and a nail-like teleoconch entirely covered by mantle shield (for zonitids: Riedel, 1980; for testacellids: Quick, 1960; for parmacellids: Solem, 1979). The Nearctic zonitid *Vitrinizonites* has a dorsal shell but lacks the mantle shield and spatulate right shell lobe (Pilsbry, 1946). The Afrotropical urocyclids *Gymnarion*, *Africarion*, etc. have a large dorsal shell partly covered by mantle shield, but a truncated tail with caudal fossa and horn (Schileyko, 2002).

Description

Body snail-like (species of *Guerrina*, *Oligolimax* and *Trochovitrina*), semislug-like (most vitrinids) or slug-like (only *Plutonia atlantica*). Snail-like vitrinids have shell large enough to withdraw body into it completely; on the contrary, semislug-like vitrinids have shell reduced so that they cannot withdraw body into it completely. Slug-like *Plutonia* has an internal limacella-like shell.

Mantle edge usually with one or two latero-posterior shell lobes, one more or less triangular right body lobe and one left – dorsal body lobe extending anteriorly to give rise to large to very large dorsal mantle shield (shell lobes absent and mantle shield very small in *Guerrina*, *Oligolimax* and *Trochovitrina*). Right shell lobe always more developed than left shell lobe (when present), more or less spatulate and covering a more or less wide portion of dorsal surface of shell; left shell lobe usually consisting of narrow strip or absent (in re-descriptions of genera, only the right shell lobe is considered, because available images of living specimens only depict their right side – cf. Kerney & Cameron, 1979; Bogon, 1990; Falkner, 1990, 1992 – and in the preserved material shape and size of shell lobes are modified due to fixation).

Foot aulacopod and sole tripartite with distinct longitudinal furrows meeting a short distance from tail tip. Posterior part of foot, below shell, with dorsal flat triangular area bordered by two raised crests which meet at about half tail length; raised keel originates at this point and ends at tail tip.

Jaw oxygnathous except in *Plutonia*, which has very arched jaw without central outgrowth.

Radula dichoglossan except in *Plutonia* where it is beloglossan (Jungbluth et al., 1985); structure of marginal teeth variable with mono- or pluricuspid teeth inside species of same genus.

Shell very thin and fragile, consisting of few whorls (never more than four) and ranging in shape from globular-conical (*Oligolimax*) to globular-depressed (*Vitrina*), conical-depressed (*Guerrina*, *Trochovitrina*) or depressed (*Arabivitrina*, *Eucobresia*, etc.). Vitrinid shells differ in shape of last whorl and width of aperture: last whorl expanded and aperture moderately wide in typical vitriniform and trochiform shells (they differ in periphery, which is rounded in typical vitriniform shells, see *Vitrina*, etc., and angled or keeled in trochiform shells, see *Guerrina* and *Trochovitrina*); last whorl well expanded and aperture wide in slightly dilated vitriniform shells (*Arabivitrina*, *Canarivitrina*, etc.); last whorl extremely expanded and aperture very wide in very dilated vitriniform shells (*Semilimax*, *Vitrinobrachium*, etc.). Columellar and basal margins of aperture with variably wide membranous border ("periostracal fringe"; absent in *Guerrina* and *Trochovitrina*). In some species (*Eucobresia diaphana*, *Hessemilimax kotulae*, etc.), columellar margin more or less cut-away so that internal winding of spire is visible. Umbilicus closed or punctiform; when columellar margin cut-away umbilical area is open. Protoconch usually smooth, sometimes more or less evidently ribbed; in both cases mineralized part, below periostracum, more or less evidently and variably pitted (five different types recognized by Ibáñez et al., 1987); sometimes pits ("puncta") few and sparse, but usually numerous and arranged in many spiral rows. Teleoconch usually smooth, sometimes with fine, closely and regularly spaced radial riblets (*Guerrina*, some *Oligolimax* and *Trochovitrina*).

Proximal genitalia (those including parts close to the gonad: first hermaphrodite duct, talon or seminal receptacle - fertilization pouch complex, albumen gland, second hermaphrodite duct or ovispermiduct) do not show significant variation between the various taxa, whereas distal genitalia have very complex and diverse structure.

Female distal genitalia include: free oviduct, bursa copulatrix and its duct and vagina. Free oviduct variably long and wide. Bursa copulatrix ("bursa of receptaculum seminis", Forcart, 1959; "gametolytic gland", Valido et al., 1993; "spermatheca", Hubendick, 1953; Forcart, 1959; Schileyko, 2003) usually rather small (oval, roundish or pyriform) and with variably long duct, sometimes initially flared. Vagina absent in *Vitrinobrachium*, very short/short in *Calidivitrina*, *Eucobresia*, *Hessemilimax*, *Semilimacella*, *Semilimax* and *Vitrina* and long in vitrinids with glandula amatoria. In some species of *Semilimacella* and *Vitrina*, vagina and initial part of duct of bursa copulatrix with external glandular layer imitating vaginal gland of zonitids. In *Hessemilimax*, *Semilimacella*, *Semilimax* and *Vitrina*, vagina lacks any internal structure. In *Calidivitrina* and type species of *Eucobresia* there is an internal proximal pad; in another alleged *Eucobresia* species (*E. nivalis*) there is a small conical papilla-like structure similar to that of a typical glandula amatoria (detailed description is given under remarks to *Eucobresia*). In the other vitrinids, vagina has distinct proximal and distal portions; proximal corresponding to vaginal stimulator, so called "glandula amatoria" ("stimulator", Mordan & Martins, 2001; "stimulator portion of vagina", Hausdorf, 1995; Alonso et al., 2000; "vaginal gland", Hubendick, 1953; Mordan & Martins, 2001; "vaginal stimulator", Muratov, 1999). Glandula amatoria is sort of wide cylinder with muscular walls covered externally and internally by glandular tissue layer; external glandular layer may partially or completely cover muscular wall and may extend to distal free oviduct and beginning of bursa copulatrix duct; its secretion is conveyed into vaginal lumen through very thin channels in muscular wall; internal glandular layer is always complete and defines narrow lumen. Distally, glandula amatoria has variably developed conical papilla ("spout", Ibáñez et al., 2001; "stimulatory papilla", Mordan & Martins, 2001; "vaginal papilla", Forcart, 1957), open at tip, protruding into distal vagina. Final part of proximal vagina is usually invaginated inside distal vagina; externally, level with initial part of invaginated portion, walls of distal vagina are connected to those of glandula amatoria by thin stripes of muscular tissue which presumably prevent evagination. *Trochovitrina* apparently lacks papilla of glandula amatoria. Distal vagina has very thin, subtransparent walls, sometimes with sparse traces of glandular tissue; internal surface of distal vagina with regularly or irregularly arranged pleats and sometimes longitudinal pilasters (*Canarivitrina*, *Oligolimax*) or laminar crests (*Plutonia*).

Male distal genitalia include vas deferens and penial complex (epiphallus absent). Vas deferens of variable length (long in *Hessemilimax*, *Phenacolimax*, *Semilimacella*, *Semilimax*, *Vitrina* and *Vitrinobrachium*; short to medium in the other genera). It may enter penial complex apically/subapically (*Plutonia* and *Sardovitrina*) or medially to distally, thus distinguishing short to long flagellum and penis proper (*Calidivitrina*, *Canarivitrina*, *Guerrina*, *Madeirovitrina* and *Trochovitrina*). Other times vas deferens contacts penial complex about medially, then traverses penial sheath (if present), running along or inside thickness of penial gland and entering penial complex subapically near insertion of penial retractor (*Arabivitrina*, *Eucobresia*, *Hessemilimax*, *Insulivitrina*, *Oligolimax*, *Phenacolimax*, *Vitrina* and *Vitrinobrachium*).

Penial complex ("penis", most authors) is very variable in shape and size: very small (e.g., *Oligolimax* and *Sardovitrina*) to large (*Canarivitrina*, *Eucobresia*, etc.), finger-like (*Canarivitrina*, *Vitrina*, etc.), club-like (*Calidivitrina*, *Eucobresia*, etc.), nearly bilobate (*Arabivitrina* and *Plutonia*) or more or less globular (*Sardovitrina*, *Vitrinobrachium*, etc.) and may have or not have a penial gland and a penial sheath. Penial gland ("apical gland", Hausdorf, 1995, 2002) is an external layer of glandular tissue covering most of penial complex (*Eucobresia*, *Phenacolimax*, etc.), or most of proximal penial complex (*Madeirovitrina*, *Oligolimax*, etc.), or part of side of penial complex facing vagina (*Arabivitrina*, *Insulivitrina*, etc.), or forming sparse glandular covering (*Sardovitrina*). Penial sheath ("penial tunica", Hausdorf, 1995, 2002) is a thin short to long sac present in *Eucobresia*, *Hessemilimax*, *Phenacolimax*, *Semilimacella*, *Semilimax*, *Vitrina*, *Vitrinobrachium* and in some species of Macaronesian vitrinids (*Azorivitrina finitima*, *Insulivitrina blauneri* and *I. lamarckii*). It may arise from distal constriction and envelope most of penial complex or from a mid constriction and envelope proximal penial complex only. It is basally traversed by vas deferens on one side (in oxychiline zonitids it is traversed apically), apically open and often wrinkled.

Penial retractor muscle arising from diaphragm (all genera except *Vitrinobrachium*) or columellar muscle (*Vitrinobrachium*) and ending on penial complex apically or subapically. Penial retractor modified in *Plutonia atlantica* being subdivided into three branches, none of which reaches penial complex: first reaches bursa copulatrix and its duct, second reaches free oviduct and third reaches vas deferens (sometimes thin muscular branches connect distal vas deferens and penial complex). Muscle bundles connecting free oviduct to duct of bursa copulatrix also present in *Madeirovitrina marcida* and *M. nitida* although these species have normal penial retractor. Finally, penial rectractor absent in two species of *Semilimax* (*S. semilimax* and *S. pyrenaicus*) and absent or vestigial in one species of *Azorivitrina* (*A. finitima*).

Internal stuctures of penial complex variable in shape, size and number: they may consist of main pilaster only (*Calidivitrina*, *Semilimacella*, *Semilimax*, *Trochovitrina* and *Vitrina*), main and accessory pilasters (*Arabivitrina*, *Guerrina*, *Madeirovitrina*, *Oligolimax*, *Phenacolimax* and *Plutonia*), two distinctive pilasters possibly homologous with main and accessory pilasters (*Eucobresia* and *Insulivitrina*), branched main pilaster (*Hessemilimax*) or extremely distinctive structures (*Canarivitrina*, *Sardovitrina* and *Vitrinobrachium*). Main pilaster usually consists of large proximal "glandular roll" (Hausdorf, 1995, in *Oligolimax*), sometimes distally lamellate and sometimes with distal smooth muscular pleat, ending in or before genital atrium. Glandular roll is a sort of elongated knoll, located inside innermost penial complex, usually in correspondence with external penial gland (especially evident in those species in which penial gland forms band on one side of penial complex). This structure has been named: "Drüsenwülste der Penis" (Forcart, 1959, in *Oligolimax*), "glandular pad" (Mordan & Martins, 2001, in Azorean "*Plutonia*"), "penial glandular torus" (Forcart, 1957, in *Arabivitrina*), "penis gland" (Hubendick, 1953, in *Arabivitrina* and *Calidivitrina*) or "torus" (Alonso et al., 2000, in *Canarivitrina*). Apart from *Sardovitrina* and *Vitrinobrachium*, all the other genera have usual or modified glandular roll. In *Arabivitrina*, *Calidivitrina*, *Guerrina* and *Madeirovitrina*, distal portion of glandular roll is lamellate; in *Canarivitrina*, *Eucobresia*, *Insulivitrina*, *Oligolimax* and *Phenacolimax* lamellate portion is not evident, but distal portion is more or less pleated; in *Hessemilimax*, *Plutonia*, *Semilimacella*, *Semilimax*, *Trochovitrina* and *Vitrina* no lamellate or pleated distal portion is evident. Glandular roll may stop abruptly near penis and open into genital atrium (*Plutonia*, *Semilimacella*, etc.) or continue uninterrupted into genital atrium as muscular pleat, having more or less evidently pleated or wrinkled surface (*Guerrina*, *Madeirovitrina*, etc.).

Accessory pilaster consists of longitudinal, superficially pleated or wrinkled structure arising side by side or from one side of glandular roll, running parallel to main pilaster, then continuing forwards, ending before or inside genital atrium. Peculiar pilasters are observed in *Canarivitrina* and *Madeirovitrina*. In the former, accessory pilaster consists of wrinkled laminar structure (which suggested name "laminar crest" or "velum"; Alonso et al., 2000). In the latter (at least in species here re-examined), accessory pilaster arises on one side of glandular roll and ends near gonopore; remarkably its medial portion is detached from genital walls as free cordon. Other structures are situated close to where penis opens into genital atrium: crescent-like crest in *Semilimacella bonelli*; tongue-like structure in *Phenacolimax major*; tongue-like structure fused with tip of main pilaster in *Insulivitrina lamarckii*.

Opening of vas deferens into penial lumen is located on glandular roll (*Madeirovitrina*, *Oligolimax*, *Phenacolimax*, *Plutonia*, *Trochovitrina*), or at tip of cylindrical papilla (*Canarivitrina* and *Sardovitrina*) or in flat structure divided into two semicircular tiles (*Vitrinobrachium*). In other cases, opening of vas deferens is not visible, but probably situated level with glandular roll.

In all species examined, genital nerve arises from right pedal ganglion, extends towards distal genitalia and ends with distinct branches on walls of distal penial complex and distal vagina.

In *Hessemilimax*, *Semilimacella*, *Semilimax* and *Vitrinobrachium*, a diverticular stimulator, consisting of more or less elongated blind sac with apical portion covered by spongy glandular tissue, is associated with the distal genitalia ("accessory organ", Forcart, 1956, 1957; "appendix", Forcart, 1956, 1957; "atrial diverticulum", Alonso et al., 2000; "Begattungsarm", Künkel, 1929, 1933; "brachium copulatorium", Forcart, 1956, 1957; "copulatory gland", Hubendick, 1953; "glande femelle", Germain, 1930; "Liebesdrüse", Hesse, 1923; "penial appendage", Kerney & Cameron, 1979; "penial appendix", Kerney & Cameron, 1979; "Pfeilsack", Wagner, 1915; "sarcobelum", Schileyko, 2003; "stimulator", Hausdorf, 1998, 2002; "vaginal sarcobellum", Muratov, 1999).

In *Hessemilimax*, *Semilimacella* and *Semilimax*, a diverticular stimulator is inserted, opposite where penial complex enters one side of genital atrium (atrial stimulator) or between vagina and genital atrium (atrial-vaginal stimulator) or on one side of distal vagina (vaginal stimulator). To the contrary, in *Vitrinobrachium* it is inserted in distal penial complex and may be termed "penial stimulator". Stimulator of *Hessemilimax* and *Semilimax* has structure very similar to that of glandula amatoria, but differs in that its internal cavity is not covered by layer of glandular tissue. Detailed description of these structures is given under the relevant genera.

Genital atrium is usually long or very long, the most remarkable exception being *Phenacolimax major* which has a very short genital atrium. In most cases, the vaginal side or both sides of genital atrium have many variably robust muscular bundles connecting them to body wall.

Relationships between right ommatophore retractor, penial retractor and distal genitalia are different in various taxa and sometimes age-dependent (Figs. 3–11). In juveniles and adults of *Oligolimax*, *Sardovitrina*, *Semilimacella* and *Vitrina* penial retractor is long and passes above the right ommatophore retractor, leaving it free of penial complex and vagina. In juveniles and adults of *Semilimax carinthiacus*, *Hessemilimax kotulae, Vitrinobrachium* and all Macaronesian vitrinids (except *Plutonia*) and in juveniles of *Arabivitrina*, *Eucobresia* and *Phenacolimax*, penial retractor is long and passes below right ommatophore and in front of right optic nerve; distal genitalia lie right of right ommatophore retractor which therefore appears free of penial complex and vagina (actually it is free only because the genitalia lie right of the right ommatophore retractor). In adults of *Arabivitrina*, *Eucobresia* and *Phenacolimax*, penial retractor is short and passes below right ommatophore retractor and in front of right optic nerve; right ommatophore retractor is consequently shifted to right and compelled to lie more or less between the penial complex and vagina.

Mating face-to-face, simultaneously reciprocal. Mating has only been described in detail in two species (*Semilimax semilimax* and *Vitrinobrachium breve*) which copulate keeping partner still by means of suckers or sucker-like structures of diverticular stimulator. In *V. breve*, sperm transfer is external (everted penial complex not inserted in vagina or duct of bursa copulatrix), whereas in *S. semilimax* it is internal (everted penial complex inserted in vagina or duct of bursa copulatrix) (Künkel, 1929, 1933). According to Hausdorf (2002), the stimulator is not used to keep partner still in *Eucobresia* and *Phenacolimax* while sperm transfer is internal in *Eucobresia* and *Vitrina* and external in *Phenacolimax* and *Plutonia* (but see remarks to these genera on *Eucobresia* and *Plutonia sensu* Hausdorf, 2002).

Vitrinids with Vaginal Stimulator (glandula amatoria)

The vitrinids with glandula amatoria include most taxa of the genus group described in the vitrinids. They are characterized by the vaginal stimulator; as it is a unique and complex structure, it presumably only appeared once in the evolution of the group. MP analysis found that the glandula amatoria is a synapomorphy of the family (Table 3).

Arabivitrina Thiele, 1931

Type Species: *Vitrina arabica* Thiele, 1910, by monotypy.

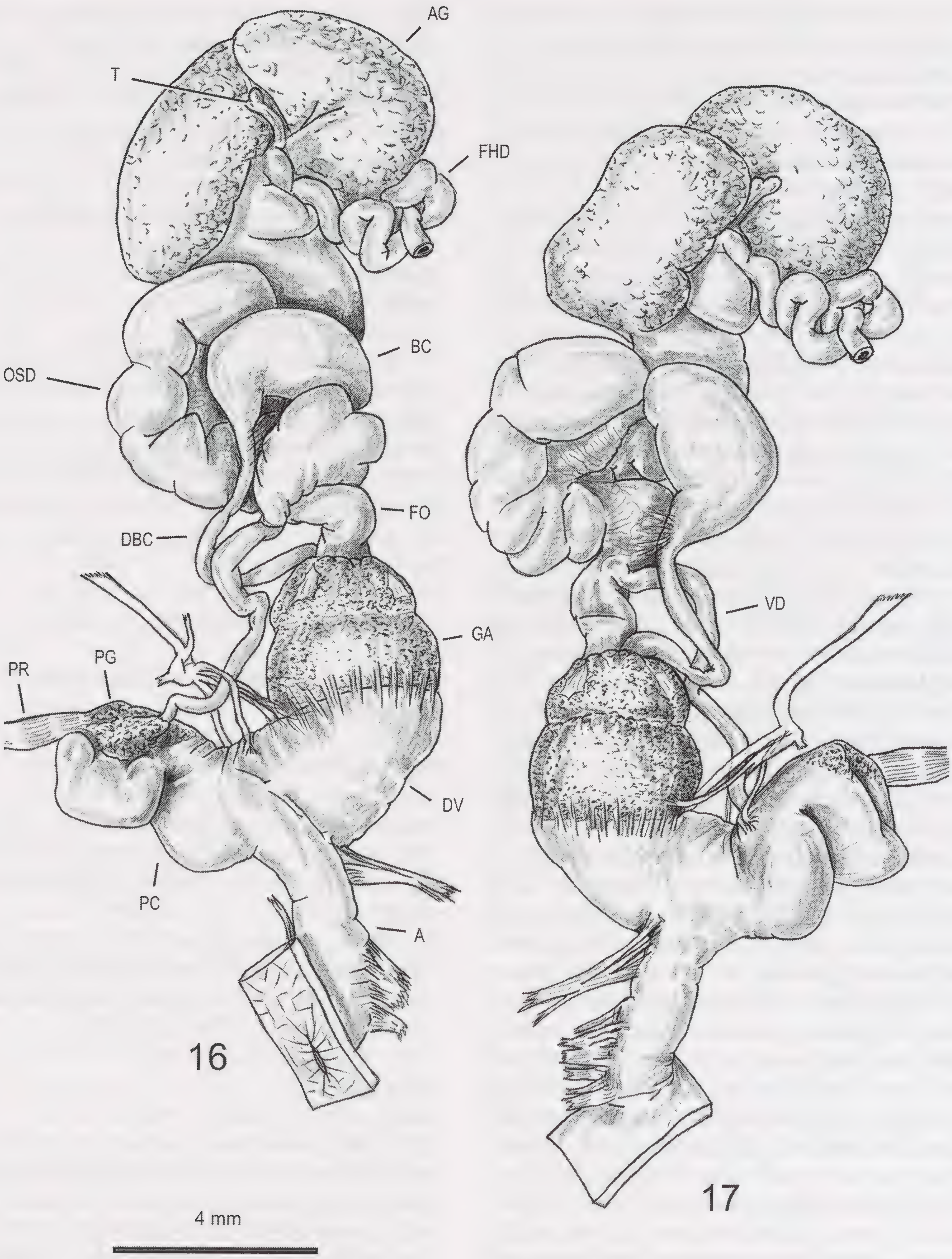
Material Examined

Arabivitrina arabica (Thiele, 1910) – Saudi Arabia: Rayah escarpment ('Asīr), P. Symens & M. Werner leg. 29.1.1994 (3 spirit specimens, SMF 311595)

Arabivitrina jansseni Neubert, 1998 – Saudi Arabia: 15 km S of Bani Sa'ad Sof Ta'if ('Asīr), R. Janssen & E. Neubert leg. 2.6.1995 (1 spirit specimen, SMF 311607).

Diagnosis

Semislug-like vitrinids characterized by short penial retractor muscle arising from diaphragm compelling penial complex below right ommatophore and in front of right optic nerve and ending on penial complex subapically (but juveniles with long penial retractor muscle running in front of right optic nerve below right ommatophore retractor); stimulator consisting of glandula amatoria with complete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens contacting penial complex about medially, running inside its wall and entering it subapically; internal structures of penial complex comprising main and accessory pilasters; main pilaster consisting of large, distally lamellate, proximal glandular roll.



FIGS. 16, 17. Distal genitalia of a specimen of *Arabivitrina arabica* (Thiele, 1910) from Rayah escarpment (ʻAsīr, Saudi Arabia), P. Symens & M. Werner leg. 29.1.1994.

Description of Type Species

Shell: vitriniform slightly dilated, glossy, thin and fragile, depressed, with 2.5–3.0 whorls; last whorl well expanded; aperture wide with basal margin bordered by narrow periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch with blunt radial ribs (Neubert, 1998: figs. 88–90).

Body: Hesse (1923: 136) described shell lobes but not dimensions. Neubert (1998: 388, fig.

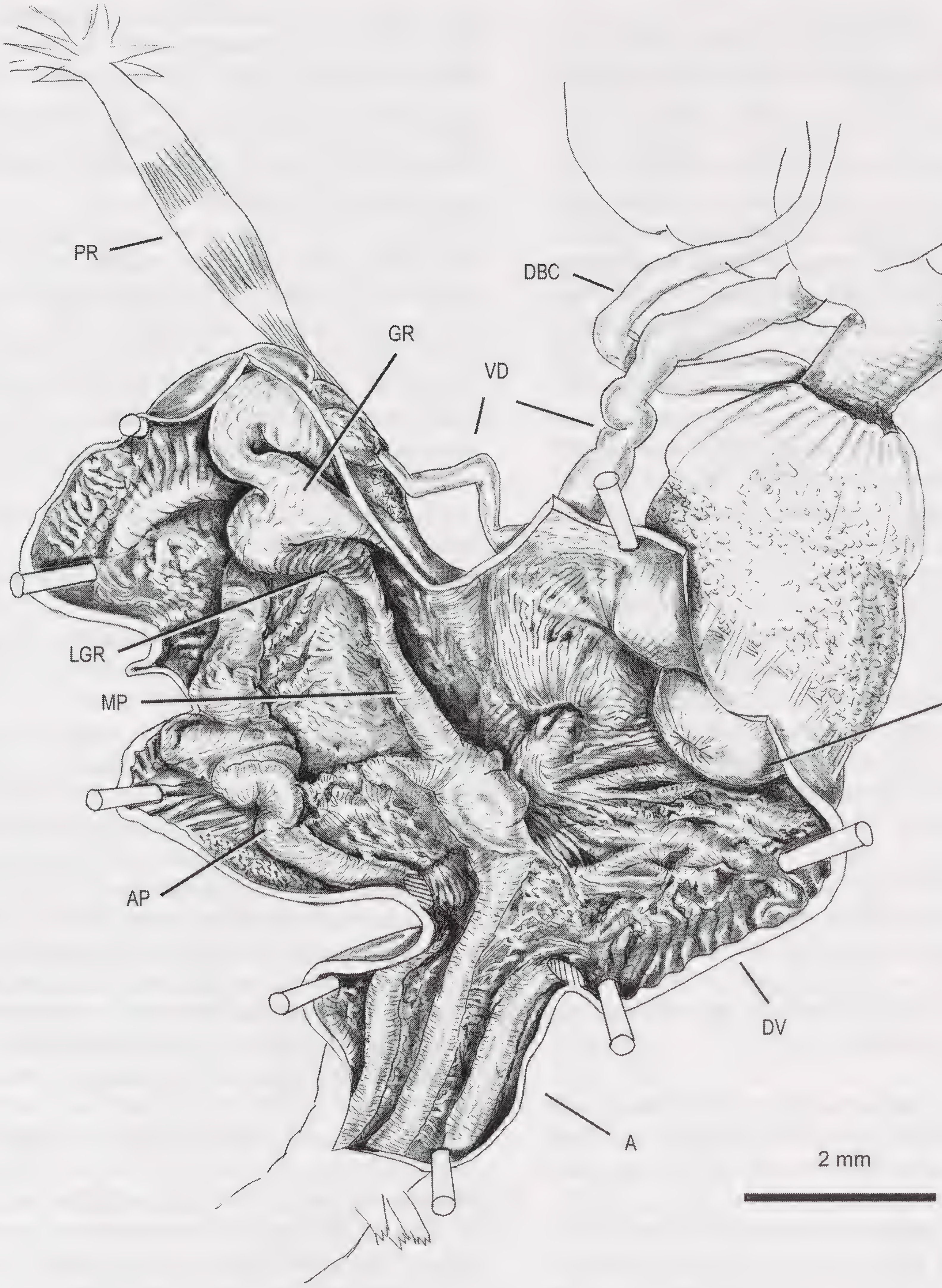


FIG. 18. Internal structure of distal genitalia of a specimen of *Arabivitrina arabica* (Thiele, 1910) from Rayah escarpment (ʻAsīr, Saudi Arabia), P. Symens & M. Werner leg. 29.1.1994.

101) stated that another *Arabivitrina* species, *A. jansseni*, cannot withdraw body completely into shell and that right shell lobe is small.

Radula: dichoglossan. Hesse (1923: 137) stated that marginal teeth are bicuspid.

Female Distal Genitalia (Figs. 16–18): free oviduct short and wide. Bursa copulatrix medium in size and oval to shoe-like in shape, with medium to long, very slender duct, initially not flared. Vagina long, with large bulbous glandula amatoria constituting half its length. Glandula amatoria with very thick muscular wall, almost completely covered by external glandular layer; papilla of glandula amatoria very short. Internal surface of distal vagina with thin pleats irregularly arranged.

Male Distal Genitalia (Figs. 16–18): penial complex small and nearly bilobate, with penial gland and without penial sheath. Penial gland covering side of proximal penial complex facing vagina. Penial retractor arising from diaphragm, short, inserted subapically. Vas deferens medium in length, contacting penial complex about medially, then running inside thickness of penial gland and entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex comprising main and accessory pilasters. Main pilaster consisting of large proximal glandular roll ("penial gland"; Neubert, 1998: fig. 97), initially slender, sometimes twisted on itself, then wider and distally lamellate ("lamellate pilaster"; Neubert, 1998: fig. 97) and medial–distal superficially wrinkled slender muscular pleat ("main pilaster"; Neubert, 1998: fig. 97), ending in genital atrium. Accessory pilaster ("longitudinal pilaster"; Neubert, 1998: 387) slender, arising near lamellate portion of glandular roll and ending in genital atrium, sometimes branching into two. Vas deferens opening not visible, probably on side of glandular roll. Internal surface with low irregularly arranged pleats and sparse spongy tissue with crypts.

Genital Atrium (Figs. 16–18): long and cylindrical, with one side connected to body wall by robust muscular bands. Internal structures consisting of short pilaster and distal portion of main and accessory pilasters. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: age-dependent (Figs. 3, 6). Juveniles (Fig. 3) with long slender penial retractor passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina. Adults (Fig. 6) with penial retractor short so that entire penial complex is below right ommatophore and in front of right optic nerve; right ommatophore is consequently shifted to right and compelled to lie between penial complex and vagina.

Mating: no data available.

Remarks

Arabivitrina was introduced by Thiele (1931) as a section of the subgenus *Vitrina (Insulivitrina)*. Later, Hubendick (1953) regarded it as a junior synonym of *Vitrina (Phenacolimax)* and Forcart (1957, 1978) as a sugbenus of *Phenacolimax*. Recently, Schileyko (1986, 2003), Neubert (1998), Alonso et al. (2000) and Hausdorf (2002) accepted *Arabivitrina* as a distinct genus. Neubert (1998) reinvestigated the type species and concluded that *Arabivitrina* was close to *Insulivitrina*.

Hausdorf (2002) assigned seven species from SW Arabia, Abyssinia, Kenya and Uganda to *Arabivitrina* (major anatomical references on which are: Hesse, 1923; Thiele, 1933; Hubendick, 1953; Forcart, 1957; Neubert, 1998). He found that *Arabivitrina* was the sister group of *Calidivitrina* and that *Arabivitrina* plus *Calidivitrina* were, in turn, the sister group of *Plutonia* plus *Oligolimax*.

Only a few of the seven species assigned to *Arabivitrina* (Hausdorf, 2002) are known in detail anatomically. In most cases, available data only concerns the general scheme of the genitalia, so that major features of the internal structure of the penis and glandula amatoria are unknown or insufficiently described.

Besides the type species, we studied another species anatomically: *A. jansseni* from the Arabian peninsula. Apart from conchological differences (shell more globose, last whorl less dilated, aperture subcircular), it differs from the type species in minor anatomical details (free oviduct long and slender vs. very short and wide; vagina very long vs. vagina long; glandula amatoria one quarter of vagina length vs. half vagina length; penis long and slender vs. rather short). No substantial differences in internal structure of the penis were found in the two species

This group of vitrinids is relatively unknown: since many species have only been studied summarily, a thorough survey is needed. Moreover, there is no clear evidence that it is a monophyletic group, although BI analysis recovered the two species of this genus examined (*A. arabica* and *A. jansseni*) as a monophyletic group on the basis of one, non exclusive synapomorphy: the absence of a penial sheath (Table 3).

***Azorivitrina*, new genus**

Type Species: *Vitrina laxata* Morelet, 1860.

Material Examined

Azorivitrina brevispira (Morelet, 1860) – Portugal: Ilha de Santa Maria (Azores), Pico Alto, A. M. F. Martins leg. 9.10.1975 (3 spirit specimens, FGC).

Azorivitrina brumalis (Morelet, 1860) – Portugal: Ilha de São Miguel (Azores), Agua d'Alto, A. M.

F. Martins leg. 23.9.1970 (9 spirit specimens, FGC); Ilha de Pico (Azores), Cabeço da Urze, A. M. F. Martins leg. 13.2.1995 (5 spirit specimens, FGC).

Azorivitrina finitima (Morelet, 1860) – Portugal: Ilha de Flores (Azores), Ribeira dos Ilhéus, A. M. F. Martins leg. 16.10.1993 (1 spirit specimen, FGC).

Azorivitrina laxata (Morelet, 1860) – Portugal: Ilha de São Miguel (Azores), Falca, Santa Iria, A. M. F. Martins leg. 23.10.2006 (3 spirit specimens, FGC); Ilha de São Miguel (Azores), Porto Formoso, A. M. F. Martins leg. 23.11.2005 (3 spirit specimens, FGC); Ilha de São Miguel (Azores), Sete Cidades, Cumieira, A. M. F. Martins leg. 7.12.2005 (1 spirit specimen, FGC).

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically; stimulator consisting of glandula amatoria (absent in *A. brevispira*) with complete external glandular covering; penial complex with penial gland and without penial sheath (present in *A. finitima*); vas deferens entering penial complex medially or subapically (in *A. finitima*); internal structures of penial complex comprising main and accessory pilasters; main pilaster consisting of proximal glandular roll, distally lamellate or otherwise; accessory pilaster proximally funnel-like or pouch-like.

Description of Type Species

Shell: vitriniform slightly dilated, glossy, thin and fragile, depressed, with approximately 3.0 whorls, last whorl expanded; aperture wide with columellar and basal margins bordered by narrow periostracal fringe; columellar margin normal; umbilicus open, punctiform; protoconch slightly prominent, smooth, with many regular spiral rows of very small pits; teleoconch with faint radial growth lines (Mordan & Martins, 2001: figs. 2C, 7A–F).

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed (Mordan & Martins, 2001: fig. 8A).

Radula: dichoglossan; marginal teeth bicuspid (Mordan & Martins, 2001: 355).

Female Distal Genitalia (Figs. 19, 20): free oviduct short and wide. Bursa copulatrix small and oval, with short to medium, more or less slender duct, initially not flared. Vagina with large oval glandula amatoria constituting about one third of its length. Glandula amatoria with very thick muscular wall, completely covered by external glandular layer; papilla of glandula amatoria short. Internal surface of distal vagina with longitudinal pleated pilaster starting from base of papilla of glandula amatoria and ending near genital atrium and low, sparse, mainly transverse pleats.

Male Distal Genitalia (Figs. 19, 20): penial complex small and club-like, with penial gland and without penial sheath. Penial gland partly covering side of proximal penial complex facing vagina. Penial retractor arising from diaphragm, long, inserted subapically. Vas deferens medium in length, entering penial complex about medially, thus distinguishing short stout flagellum and penis proper. Internal structures of penial complex comprising main pilaster and accessory pilaster. Main pilaster consisting of large proximal glandular roll, distally lamellate. Accessory pilaster short, arising funnel-like on one side of glandular roll and ending in proximal genital atrium. Vas deferens opening in transverse slit on glandular roll at about half its length. Internal surface smooth.

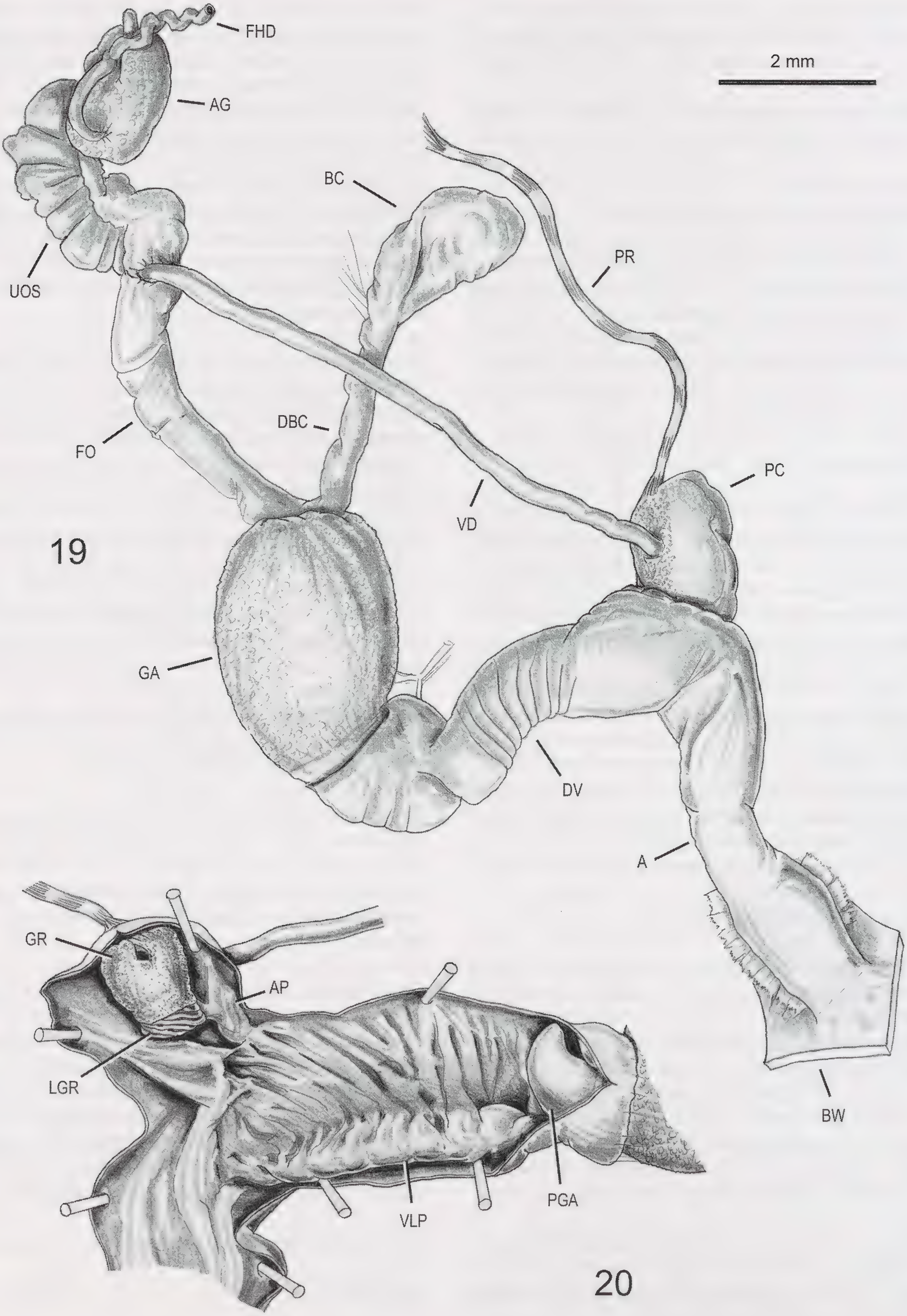
Genital Atrium (Figs. 19, 20): very long and cylindrical, with opposite sides connected to body wall by robust muscular bands. Internal structures consisting of distal portion of main and accessory pilasters. Internal surface smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 3). Penial retractor long, passing between fork formed by retractors of right ommatophore and right lower tentacle, around right ocular nerve, then backwards to end on diaphragm; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.

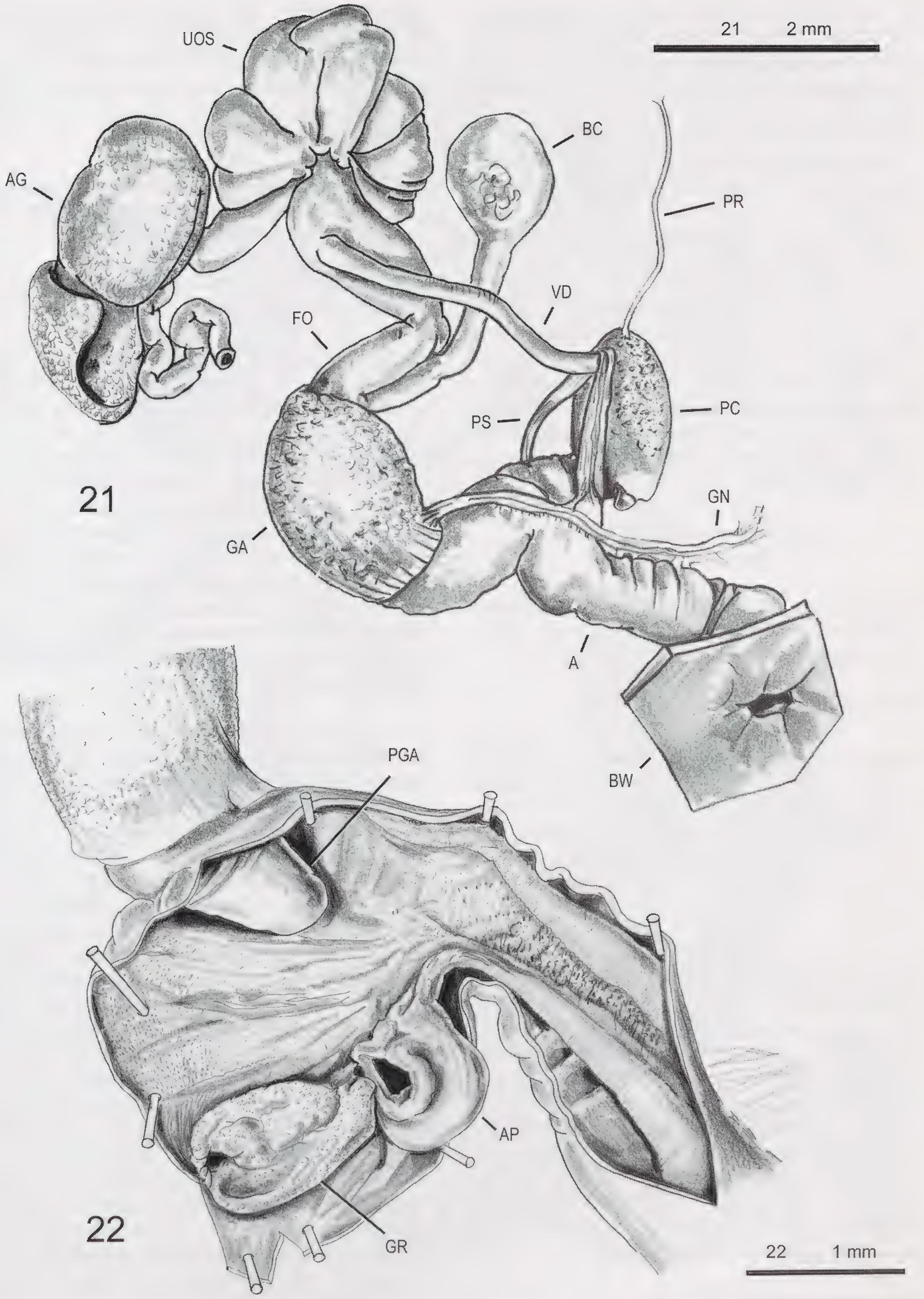
Mating: no data available.

Remarks

All four species of Azorean vitrinids reexamined by us share an accessory pilaster arising on one side (*A. laxata*) or at distal end



FIGS. 19, 20. *Azorivitrina laxata* (Morelet, 1860) from Ilha de São Miguel, Sete Cidades, Cumieira (Azores, Portugal), A. M. F. Martins leg. 7.12.2005. FIG. 19: Distal genitalia; FIG. 20: Internal structure of distal genitalia.



FIGS. 21, 22. *Azorivitrina finitima* (Morelet, 1860) from Ilha de Flores, Ribeira dos Ilhéus (Azores, Portugal), A. M. F. Martins leg. 16.10.1993. FIG. 21: Distal genitalia; FIG. 22: Internal structure of distal genitalia.

(*A. brevispira*, *A. brumalis* and *A. finitima*) of glandular roll with funnel-like or pouch-like structure. Phylogenetic analysis confirmed that the species of *Azorivitrina* constitute a variably supported monophyletic group (Figs. 14, 15; Table 3). We interpreted the unique structure of the accessory pilaster as a synapomorphy supporting an Azorean monophyletic group of vitrinids.

Apart from *A. brumalis*, the other *Azorivitrina* species differ noticeably from the type species. *A. finitima* is characterized by a penial complex having a slender penial sheath, a thin penial retractor (sometimes absent) and main pilaster consisting of a glandular roll, not lamellate distally (Figs. 21, 22). *A. brevispira* lacks glandula amatoria and has a large penial complex with a well developed glandular roll having a distal portion with small thorny papillae (Figs. 23, 24).

Canarivitrina Valido & Alonso in: Alonso et al., 2000

Type Species: *Plutonia (Canarivitrina) taburientensis* Groh & Valido in: Alonso et al., 2000, by original designation.

Material Examined

Canarivitrina taburientensis (Groh & Valido in Alonso et al., 2000) – Spain: La Palma Island (Canary Islands), Caldera de Taburiente, 28RBS1879, M. R. Alonso & M. Ibáñez leg. 8.1.1988 (1 spirit specimen, FGC).

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically; stimulator consisting of glandula amatoria with complete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens entering penial complex medially or distally; internal structures of penial complex distinctive and consequently difficult to homologize, comprising glandular roll, papilla-like structure, muscular pleat and laminar crest.

Description of Type Species

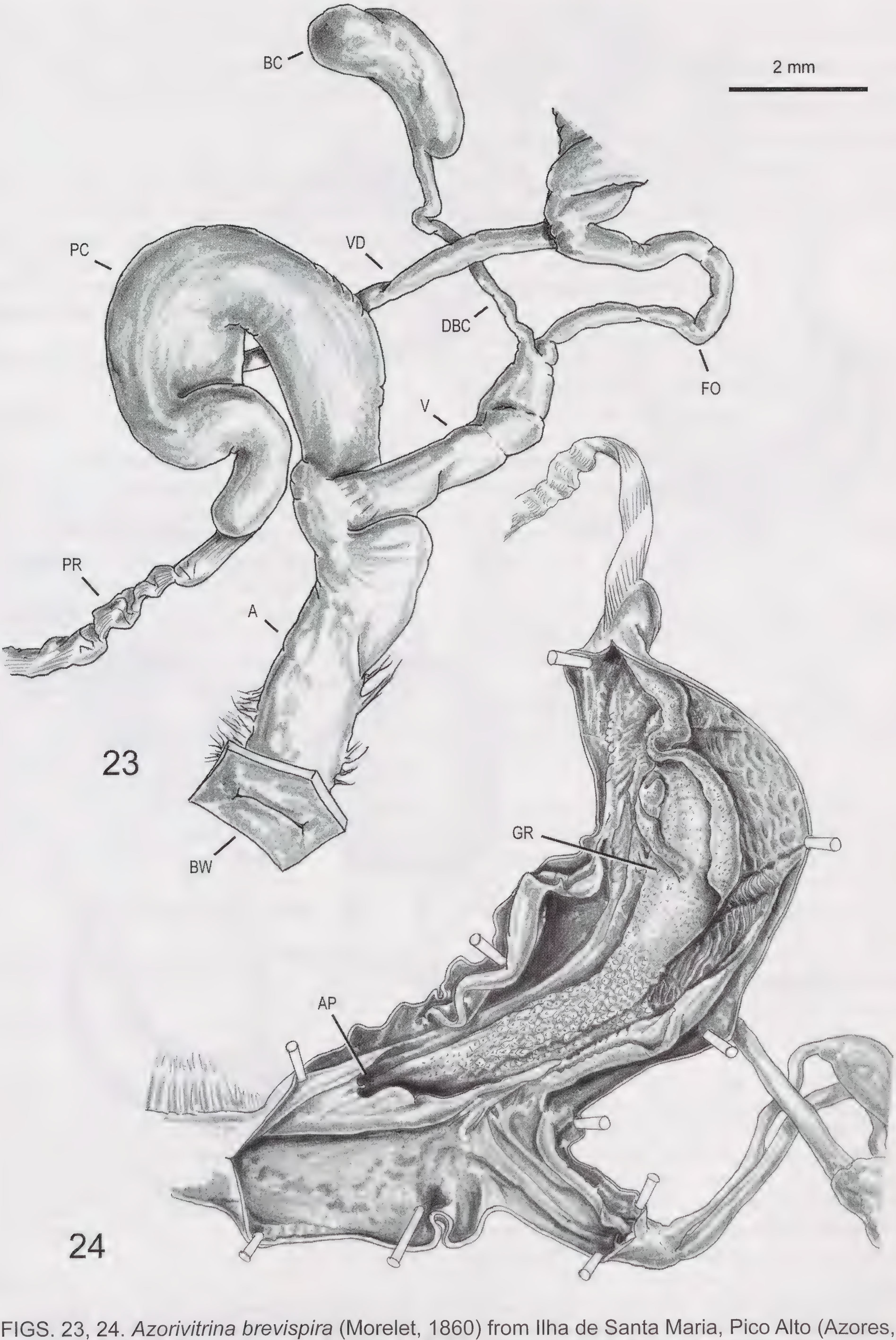
Shell: vitriniform slightly dilated, glossy, thin and fragile, depressed, with approximately 2.5 whorls; last whorl well expanded; aperture wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Alonso et al., 2000: figs. 2A, E).

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed.

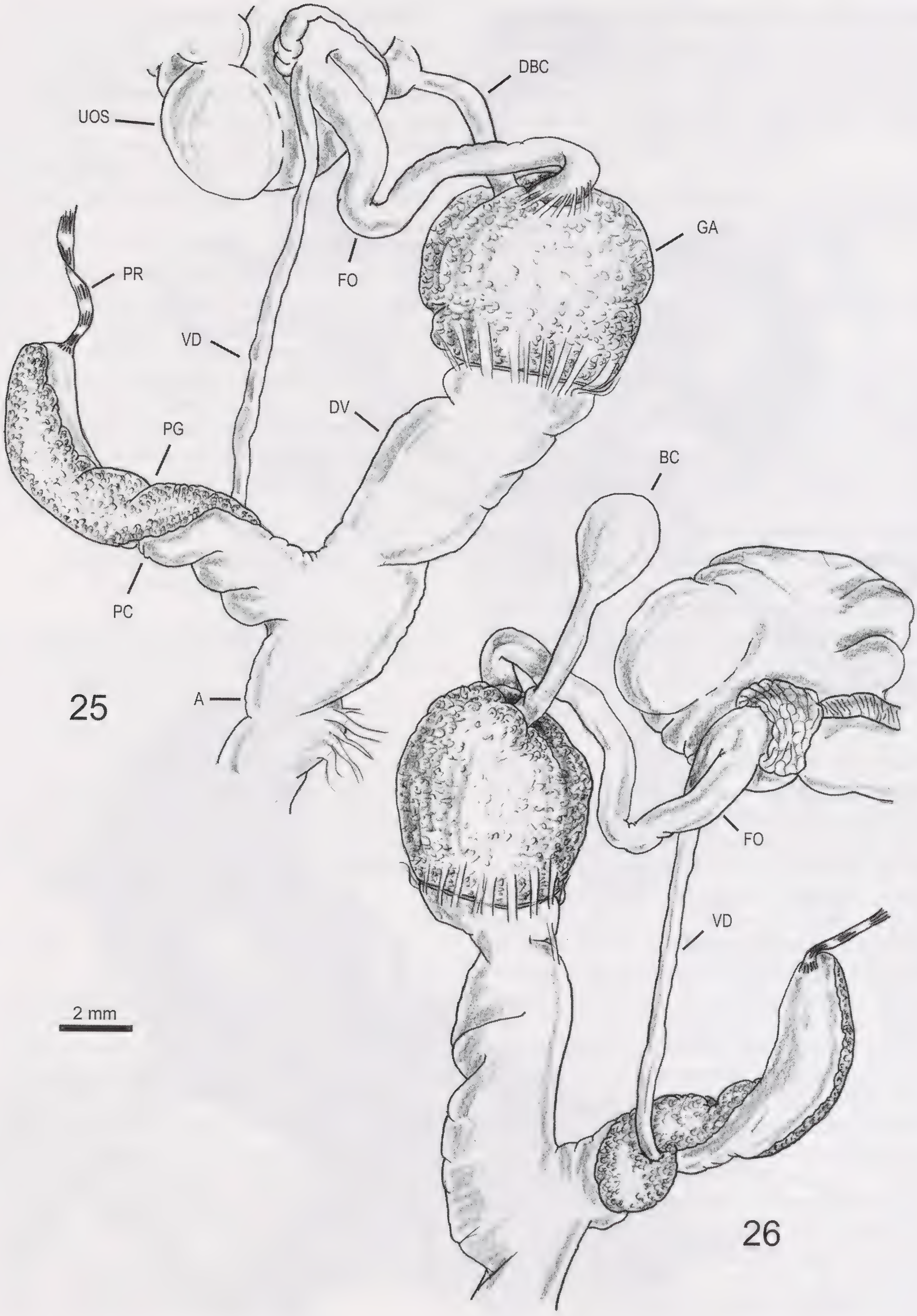
Radula: dichoglossan; marginal teeth unicuspid (Alonso et al., 2000: 50).

Female Distal Genitalia (Figs. 25–28): free oviduct long and slender. Bursa copulatrix small and oval, with short, more or less slender duct, initially not flared. Vagina long, with small bulbous glandula amatoria constituting about one third of its length. Glandula amatoria with very thick muscular walls, completely covered by external glandular layer; papilla of glandula amatoria very short. Internal surface of distal vagina with low irregular transverse pleats and two longitudinal pleated pilasters starting from base of papilla of glandula amatoria and ending near genital atrium (the shorter one) or in genital atrium (the longer one).

Male Distal Genitalia (Figs. 25–28): penial complex large and finger-like (sometimes twisted on itself), with penial gland and without penial sheath. Penial gland covering one side of penial complex. Penial retractor arising from diaphragm, very long, inserted subapically. Vas deferens medium in length, entering penial complex subdistally, thus distinguishing long pointed flagellum and short penis proper. Internal structures of penial complex distinctive, and consequently difficult to homologize, comprising glandular roll, papilla-like structure, muscular pleat and laminar crest (glandular roll - muscular pleat and laminar crest presumably homologous with main and accessory pilasters, respectively). Long slender, not distally lamellate, glandular roll ("torus"; Alonso et al., 2000) located in correspondence with penial gland and ending at base of small cylindrical papilla-like structure. Short, superficially pleated muscular pleat arising distally in respect to papilla-like structure and ending in proximal genital atrium. Long, thin, rolled, laminar crest ("velum"; Alonso et al., 2000) running parallel on opposite side to glandular roll. Vas deferens opening at tip of papilla-like structure. Internal surface with thin, mainly transverse pleats and diffuse spongy glandular tissue with crypts or smooth.



FIGS. 23, 24. *Azorivitrina brevispira* (Morelet, 1860) from Ilha de Santa Maria, Pico Alto (Azores, Portugal), A. M. F. Martins leg. 9.10.1975. FIG. 23: Distal genitalia; FIG. 24: Internal structure of distal genitalia.



FIGS. 25, 26. Distal genitalia of a specimen of *Canarivitrina taburientensis* (Groh & Valido in: Alonso et al., 2000) from La Palma Island, Caldera de Taburiente (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 8.1.1988.

Genital Atrium (Figs. 25–28): short, with one side connected to body wall by thin muscular bands. Internal structure consisting of short pilaster and distal portion of larger vaginal pilaster. Internal surface as in distal vagina.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 3). Penial retractor very long, passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.

Mating: no data available.

Remarks

Alonso et al. (2000) recently described *Canarivitrina* for five species from the Canary Islands that shared some penial characters. They also discovered that *Canarivitrina* shares relationships between penial retractor and right ommatophore retractor with the other Macaronesian vitrinids (*Guerrina*, *Insulivitrina*, *Madeirovitrina* and *Plutonia*) and therefore lumped them into a single genus (*Plutonia*), ranking *Canarivitrina* and the other genus-group taxa as subgenera.

Canarivitrina constitutes one of the best defined monophyletic groups among Macaronesian vitrinids, supported by at least one-two exclusive synapomorphies of the penial complex (Figs. 14, 15; Table 3), though its deep relationships remain unresolved.

Guerrina Odhner, 1954

Type Species: *Helix cuticula* Shuttleworth, 1852, by monotypy.

Material Examined

Guerrina cuticula (Shuttleworth, 1852) – Spain: Tenerife Island (Canary Islands), Barranco del Agua, M. R. Alonso & M. Ibáñez leg. 12.12.1984 (2 spirit specimens, FGC); Tenerife Island (Canary Islands), El Roquillo, M. R. Alonso & M. Ibáñez leg. 24.4.1984 (2 spirit specimens, FGC).

Diagnosis

Snail-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically or laterally; stimulator consisting of glandula amatoria with incomplete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens entering penial complex medially; internal structures of penial complex comprising only main pilaster, consisting of large, distally lamellate, proximal glandular roll and distal muscular pleat.

Description of Type Species

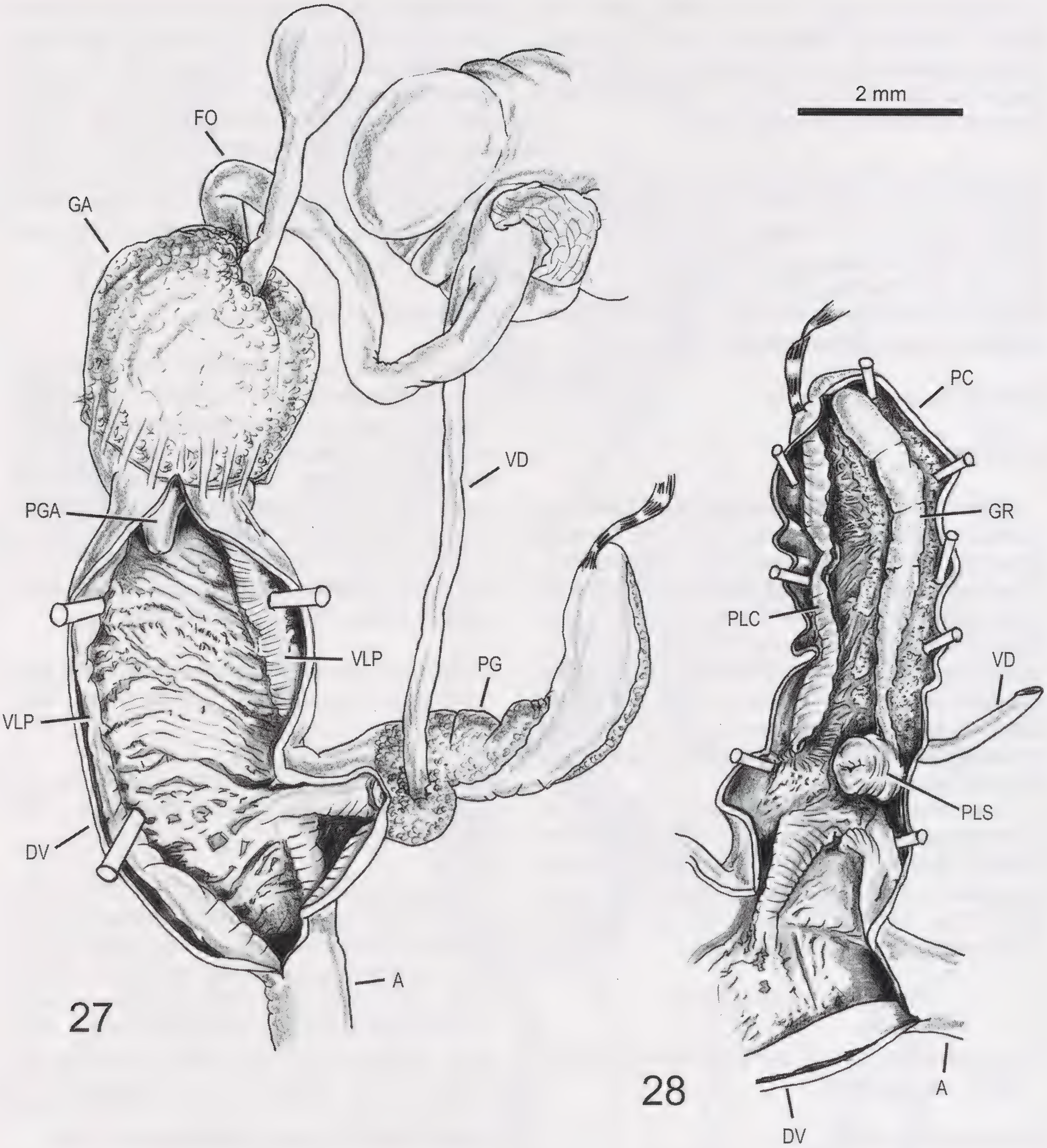
Shell: trochiform, translucent, thin and fragile, conical-depressed, sharply keeled, with approximately 3.0 whorls; last whorl expanded; aperture moderately wide with margin not bordered by periostracal fringe; columellar margin normal; umbilicus closed; protoconch prominent, with fine, closely spaced, radial riblets and large pits inbetween; teleoconch with fine, closely and regularly spaced, slightly wavy, radial riblets (Ibáñez et al., 1987: figs. 32, 38, 39).

Body: can be withdrawn completely into shell; right shell lobe absent (Ibáñez et al., 1987: fig. 3).

Radula: dichoglossan; marginal teeth unicuspid (Odhner, 1954: 57, fig. 2; Ibáñez et al., 1987: 124, fig. 19).

Female Distal Genitalia (Figs. 29–31): free oviduct short to long and slender. Bursa copulatrix small and oval to globular, with short to long, very slender duct, initially not flared. Vagina long, with small heart-like glandula amatoria constituting about one third of its length. Glandula amatoria with very thick muscular wall, covered only proximally; papilla of glandula amatoria very short. Internal surface of distal vagina with low mainly transverse pleats.

Male Distal Genitalia (Figs. 29–31): penial complex small and club-like, with penial gland and without penial sheath. Penial gland covering most of penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens medium in length, entering penial complex medially inside deep constriction, thus distinguishing short, pointed flagellum and penis proper. Internal structures of penial complex comprising main pilaster only which consists of large proximal glandular roll, distally lamellate, and smooth distal muscular pleat, ending in proximal genital atrium. Vas deferens opening

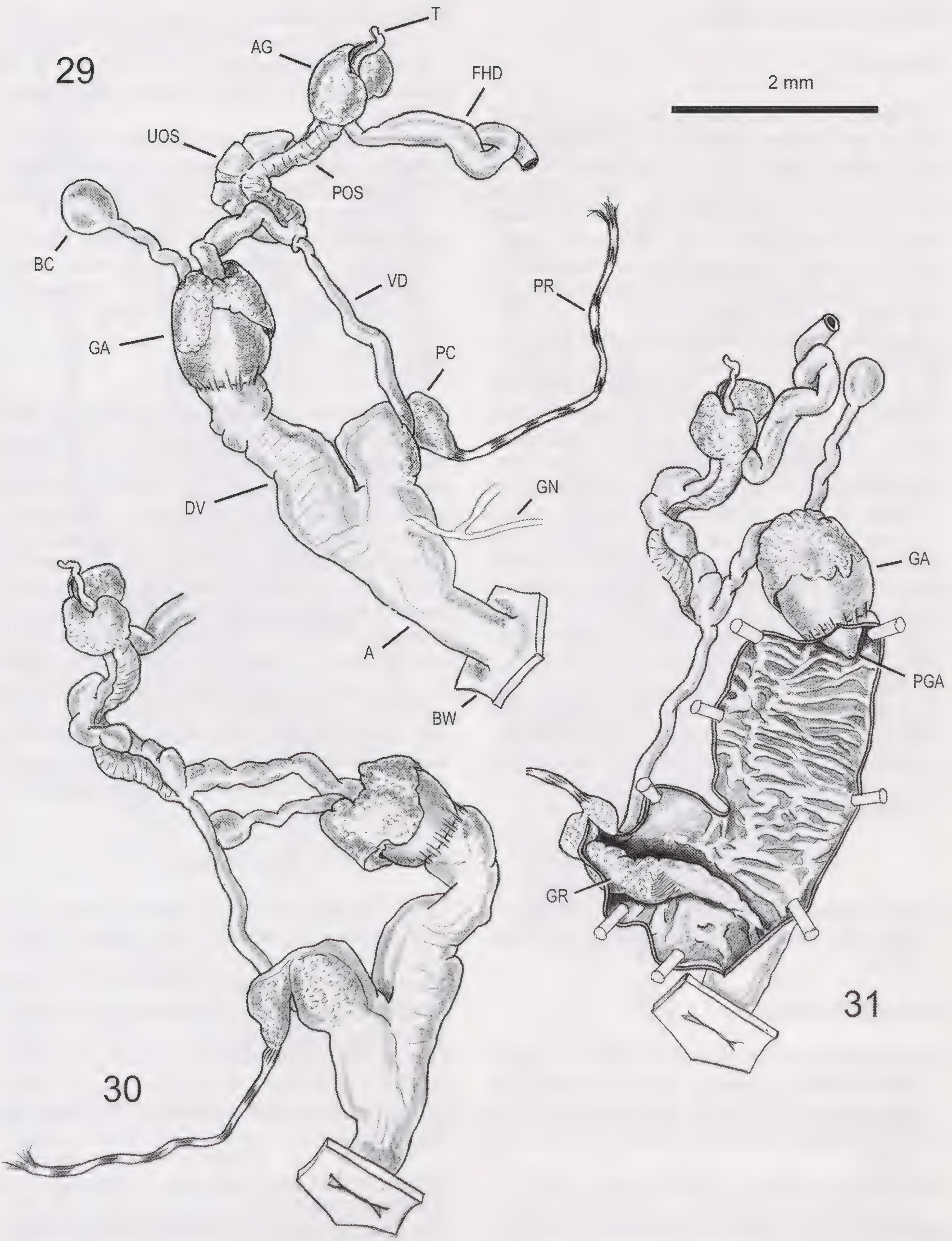


FIGS. 27, 28. *Canarivitrina taburientensis* (Groh & Valido in: Alonso et al., 2000) from La Palma Island, Caldera de Taburiente (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 8.1.1988. FIG. 27: Internal structure of vagina; FIG. 28: Penial complex.

not visible, probably level with glandular roll. Internal surface smooth.

Genital Atrium (Figs. 29–31): long and funnel-like, with one side connected to body wall by thin muscular bands. Internal structure consisting of distal portion of main pilaster. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 3). Penial retractor long, passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.



FIGS. 29–31. *Guerrina cuticula* (Shuttleworth, 1852) from Tenerife Island, Barranco del Agua (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 12.12.1984. FIGS. 29, 30: Distal genitalia; FIG. 31: Internal structure of penial complex and vagina.

Mating: no data available.

Remarks

Guerrina was established as a subgenus of *Vitrina* by Odhner (1954) for a species from the Canary Islands. Later it was regarded as a subgenus of *Phenacolimax* by Zilch (1959), a distinct genus by Schileyko (1986, 2003), Ibáñez et al. (1987) and Valido et al. (1993) and, finally, a subgenus of *Plutonia* by Alonso et al. (2000) and Hausdorf (2002) (for other comments, see Remarks on *Canarivitrina*).

Guerrina includes only two species from the Canary Islands: *G. christinae* (Groh in: Valido et al., 1993) and *G. cuticula* (major anatomical references on which are: Odhner, 1954; Ibáñez et al., 1987; Valido et al., 1993), which differ in a number of anatomical characters (penial complex with short pointed flagellum; penial retractor inserted at apex of flagellum; marginal teeth unicuspid in *G. cuticula*; penial complex with large globular flagellum; penial retractor inserted laterally half way along penial complex; marginal teeth pluricuspid in *G. christinae*; for *G. cuticula*: Figs. 29–31; Ibáñez et al., 1987; Valido et al., 1993; for *G. christinae*: Valido et al., 1993).

Alonso et al. (2000) regarded *Guerrina* as a monophyletic group based on the fact that it can withdraw into its shell. Indeed all analyses recovered *Guerrina* as monophyletic, supported by at least one non exclusive synapomorphy: snail habitus (Figs. 14, 15; Table 3).

Insulivitrina Hesse, 1923

Type Species: *Helicolimax lamarckii* Férussac, 1821, by subsequent designation (Hesse, 1924).

Material Examined

Insulivitrina lamarckii (Férussac, 1821) – Spain: Tenerife Island (Canary Islands), Vueltas de Taganana, 28RCS8158, M. R. Alonso & M. Ibáñez leg. 19.12.1988 (4 spirit specimens, FGC).

Insulivitrina blauneri (Shuttleworth, 1852) – Spain: Tenerife Island (Canary Islands), Barranco del Agua, M. R. Alonso & M. Ibáñez leg. 12.12.1984 (2 spirit specimens, FGC).

Insulivitrina canariensis (Mousson, 1872) – Spain: El Hierro Island (Canary Islands), Fuente de Mencáfete, M. J. Valido leg. 15.5.1984 (2 spirit specimens, FGC).

Insulivitrina eceroensis Alonso & Ibáñez, 1987 – Spain: El Hierro Island (Canary Islands), Fuente de Mencáfete, M. R. Alonso & M. Ibáñez leg. 15.5.1984 (3 spirit specimens, FGC).

Insulivitrina gomerensis Alonso & Ibáñez, 1988 – Spain: La Gomera (Canary Islands), Montaña Quemada, K. Groh & M. Ibáñez leg. 2.1.1988 (4 spirit specimens, FGC).

Insulivitrina oromii Ibáñez & Alonso, 1988 – Spain: La Gomera (Canary Islands), Montaña de la Zarza, K. Groh & M. Ibáñez leg. 3.1.1988 (1 spirit specimen, FGC).

Diagnosis

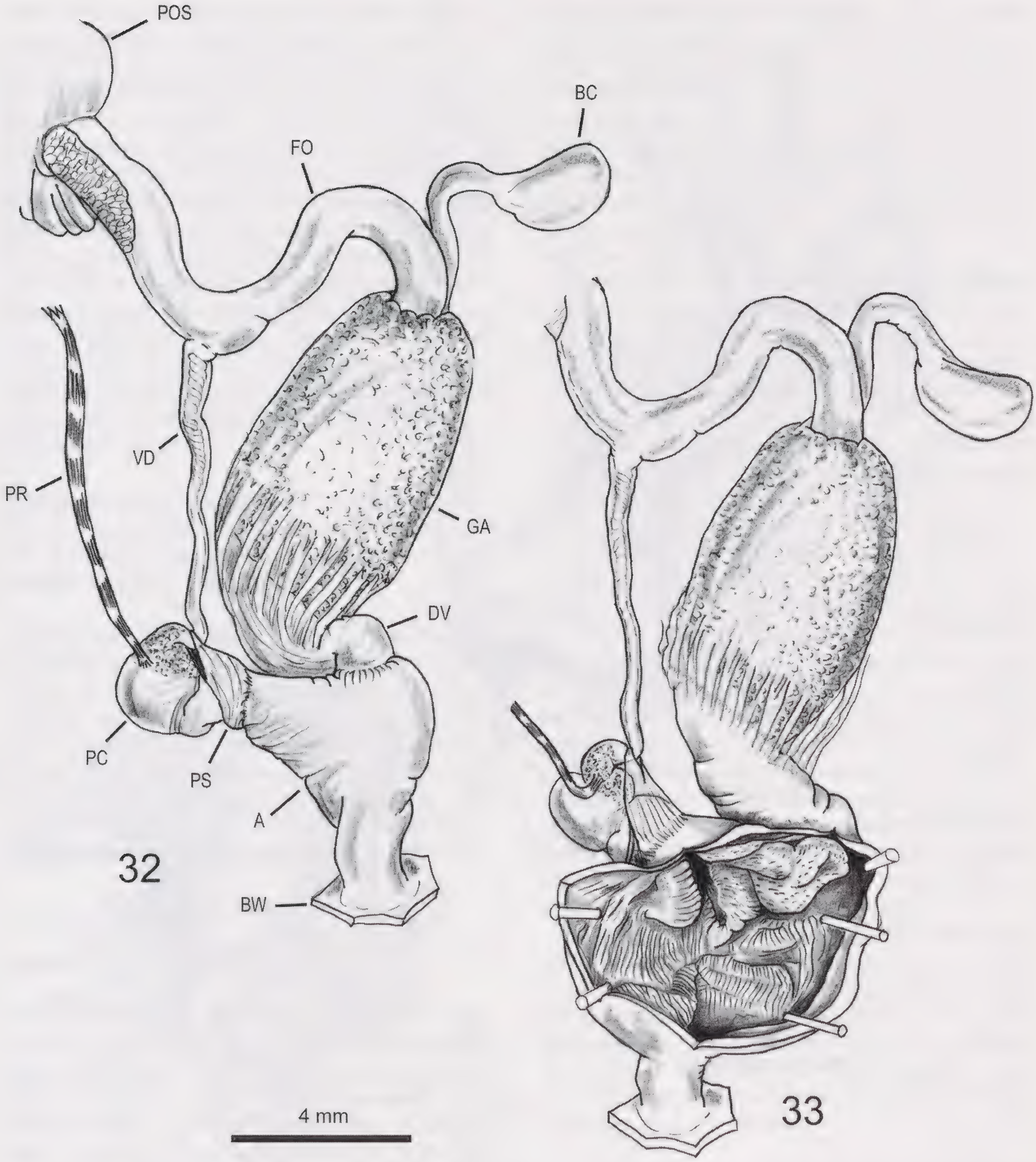
Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically; stimulator consisting of glandula amatoria with complete external glandular covering; penial complex with penial gland and reduced penial sheath (absent in many species); vas deferens contacting penial complex medially, traversing penial sheath and entering it apically or subapically (when penial sheath absent, vas deferens entering penial complex apically or subapically); internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters (at least in type species).

Description of Type Species

Shell: vitriniform very dilated, glossy, thin and fragile, depressed, with approximately 2.5 whorls; last whorl extremely expanded; aperture very wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Ibáñez et al., 1987: figs. 33, 40, 41).

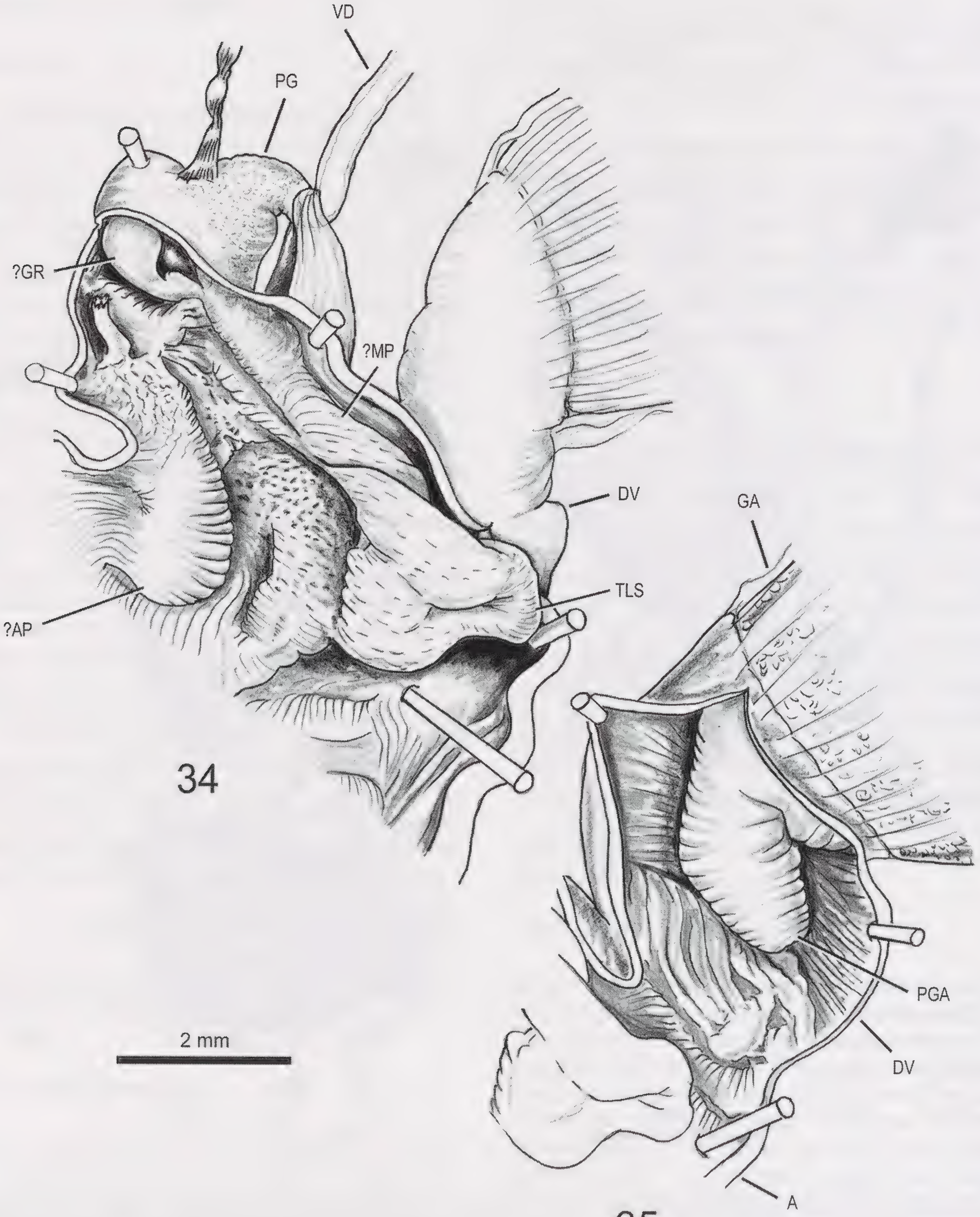
Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed (Ibáñez et al., 1987: fig. 4).

Radula: dichoglossan; marginal teeth bicuspid (with one small ectocone) (Hesse, 1923: 135, pl. 2, fig. 18; Ibáñez et al., 1987: 129, fig. 22).



FIGS. 32, 33. *Insulivitrina lamarckii* (Férussac, 1821) from Tenerife Island, Vueltas de Taganana (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 19.12.1988. FIG. 32: Distal genitalia; FIG. 33: Internal structure of penial complex.

Female Distal Genitalia (Figs. 32–35): free oviduct short to long and wide. Bursa copulatrix small and oval, with short to medium, more or less slender duct, initially not flared. Vagina long, with large oval glandula amatoria constituting about 2/3 of its length. Glandula amatoria with very thick muscular wall, completely covered by external glandular layer; papilla of glandula amatoria long. Internal surface of distal vagina with irregular low transverse pleats and sort of pad bordering opening into genital atrium.



FIGS. 34, 35. *Insulivitrina lamarckii* (Férussac, 1821) from Tenerife Island, Vueltas de Taganana (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 19.12.1988. FIG. 34: Internal structure of penial complex; FIG. 35: Vagina.

Male Distal Genitalia (Figs. 32–35): penial complex small and globular, with penial gland and penial sheath. Penial gland covering side of proximal penial complex facing vagina. Penial sheath short, arising from mid penial complex. Penial retractor arising from diaphragm, long, inserted subapically. Vas deferens medium in length, contacting penial complex medially, then traversing penial sheath and entering penial complex apically near insertion of penial retractor. Internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters. Larger pilaster (main pilaster?) consisting of proximal glandular crest (modified glandular roll?), rolled upon itself and distally transversely pleated and distal muscular pleat ending in sort of tongue-like structure bent to one side as if closing distal vagina. Smaller pleated crest (accessory pilaster?) arising near rolled crest and ending before genital atrium. Vas deferens opening encircled by rolled glandular crest. Internal surface with low, irregularly arranged pleats and sparse spongy tissue with crypts.

Genital Atrium (Figs. 32–35): long and funnel-like, with one side connected to body wall by thin muscular bands. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 3). Penial retractor long, passing between fork formed by retractors of right ommatophore and right lower tentacle, around right ocular nerve, then backwards to end on diaphragm; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.

Mating: no data available.

Remarks

Insulivitrina was introduced by Hesse (1923) for four species from the Macaronesian islands (Madeira, Azores and Canary Islands) and one from the Arabian Pensinsula. The status and relationships of *Insulivitrina* have intrigued many authors: it was regarded as a distinct genus by Hoffmann (1929), Forcart (1944) and Odhner (1954), a subgenus of *Vitrina* by Thiele (1931), a junior synonym of *Phenacolimax* by Hubendick (1953), a subgenus of *Phenacolimax* by Forcart (1957), Zilch (1959) and Backhuys (1975), again a distinct genus by Schileyko (1986, 2003), Ibáñez et al. (1987) and Valido et al. (1990, 1993), a junior synonym of *Oligolimax* by Manganelli et al. (1995) and, finally, a subgenus of *Plutonia* by Alonso et al. (2000) and Hausdorf (2002).

According to Hausdorf (2002), *Insulivitrina* includes 20 species (major anatomical references on which are: Schileyko, 1986; Alonso et al., 1987; Ibáñez et al., 1987; Morales et al., 1988; Valido et al., 1990; Mordan & Martins, 2001) all from the Azores and Canary Islands and "is a paraphyletic group from which the other, more specialized Macaronesian groups are derived" (Hausdorf, 2002: 348). However the Azorean species belong to a monophyletic group exclusive to this archipelago, at least those examined by us.

Schileyko (1986: fig. 2) studied the anatomy of a specimen from Tenerife assigned to *I. teneriffae* (Quoy & Gaimard, 1832), a junior synonym of *I. lamarckii* (see: Bank et al., 2002). Actually, this specimen belongs to another species, perhaps *I. parryi* (Gude, 1896), from Gran Canaria, or *I. canariensis*, from El Hierro, according to M. R. Alonso and M. Ibáñez (pers. comm. 26.04.1999).

Besides the type species, five other species were examined: *I. blauneri* (Figs. 36–38), *I. canariensis*, *I. eceroensis*, *I. gomerensis* and *I. oromii.* They differ from the type species only by virtue of a few minor details. Hausdorf (2002) excludes presence of a penial sheath in the species of *Plutonia* (understood as including all Macaronesian vitrinids). Actually, the penial sheath is present, albeit reduced, in the type species of *Insulivitrina* (*I. lamarckii*) and in *I. blauneri*; other *Insulivitrina* species, such as *I. canariensis* and *I. oromii*, have bundles of fibres fastening wall of distal penial complex to those of terminal part of vas deferens which might be extreme residues of a penial sheath.

There is no evidence that *Insulivitrina* species constitute a monophyletic group (Figs. 14, 15; Table 3). However, as the *Insulivitrina* species are restricted to the Canary Islands, they could be a distinct Canarian monophyletic radiation of vitrinids, separate from *Canarivitrina* and *Guerrina*, or a paraphyletic group from which the other two are derived.

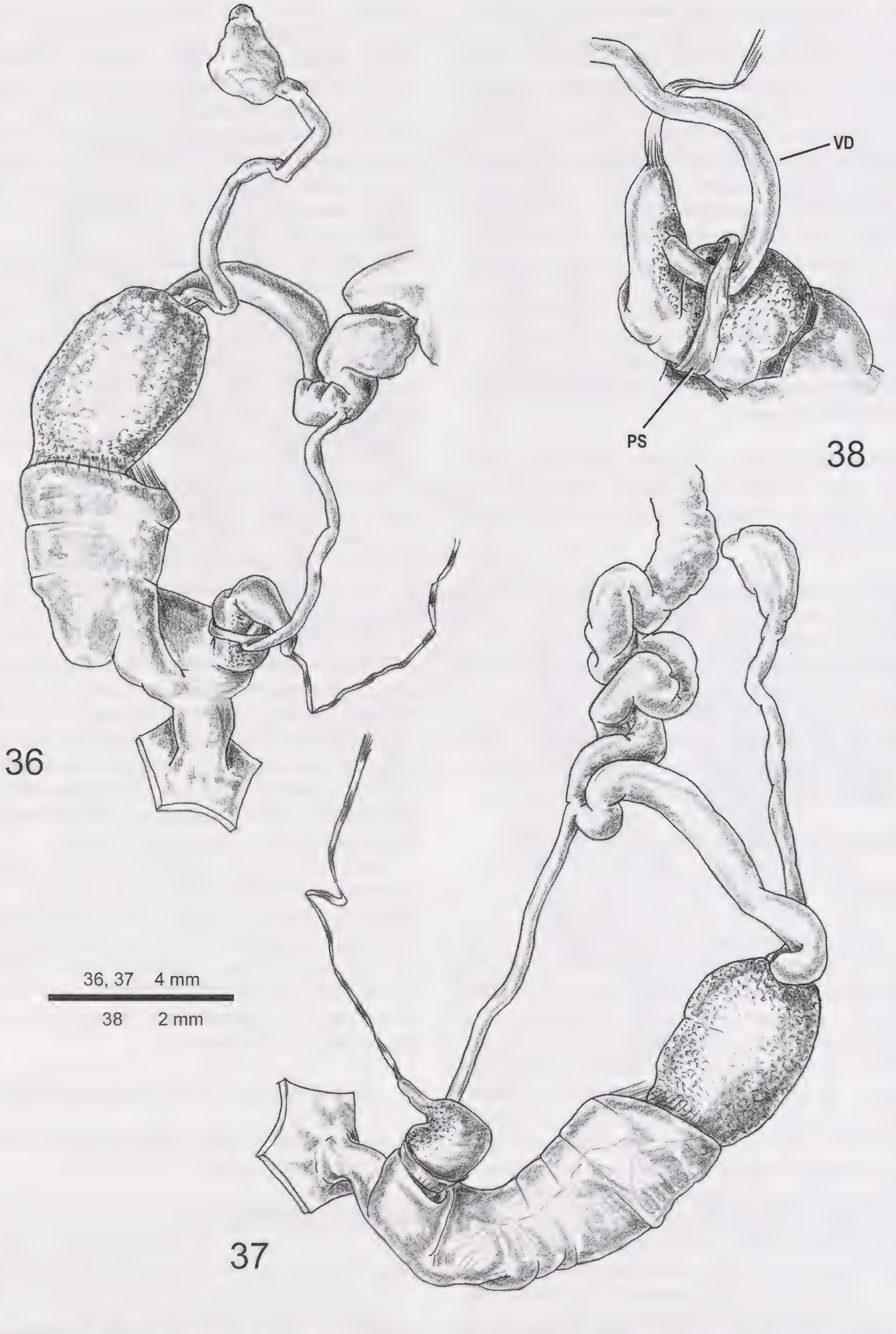
Madeirovitrina Groh & Hemmen, 1986

Type Species: *Vitrina nitida* Gould, 1847, by original designation.

Material Examined

Madeirovitrina nitida (Gould, 1847) – Portugal: Ilha da Madeira, Pico Ruivo, K. Groh leg. 7.1985 (2 spirit specimens, FGC).

Madeirovitrina marcida (Gould, 1847) – Portugal: Ilha da Madeira, Balcões, K. Groh leg. 6.1989 (2 spirit specimens, FGC).



FIGS. 36–38. *Insulivitrina blauneri* (Shuttleworth, 1852) from Tenerife Island, Barranco del Agua (Canary Islands, Spain), M. R. Alonso & M. Ibáñez leg. 12.12.1984. FIGS. 36, 37: Distal genitalia; FIG. 38: Detail of penial complex.

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically; stimulator consisting of glandula amatoria with complete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens entering penial complex medially; internal structures of penial complex comprising main and accessory pilasters; main pilaster consisting of large, distally lamellate, proximal glandular roll and smooth, robust distal muscular pleat; accessory pilaster slender, bridge-like (its medial portion detached from genital walls as free cordon).

Description of Type Species

Shell: vitriniform slightly dilated, glossy, thin and fragile, depressed, with approximately 3.0 whorls; last whorl well expanded; aperture wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Groh & Hemmen, 1986: pl. 1, figs. 1, 2).

Body: available data controversial. According to Alonso et al. (2000), *Madeirovitrina* species cannot withdraw body completely into shell and right shell lobe is well developed, covering shell almost completely when animal undisturbed. However spirit specimens of *M. nitida* and *M. marcida* have body almost completely withdrawn into shell and small right shell lobe.

Radula: dichoglossan; marginal teeth bicuspid (Groh & Hemmen, 1986: 187, pl. 5, fig. 15b).

Female Distal Genitalia (Figs. 39, 40): free oviduct short and wide. Bursa copulatrix small and oval, with short, more or less slender duct, initially not flared. Vagina long, with large cylindrical glandula amatoria constituting about 2/3 of its length. Glandula amatoria with very thick muscular wall, completely covered by external glandular layer; papilla of glandula amatoria long. Internal surface of distal vagina with low, sparse, mainly transverse pleats.

Male Distal Genitalia (Figs. 39, 40): penial complex small and club-like, with penial gland and without penial sheath. Penial gland covering most of proximal penial complex. Penial retractor arising from diaphragm, long, inserted subapically. Vas deferens medium in length, entering penial complex about medially inside groove, thus distinguishing short stout flagellum and penis proper. Internal structures of penial complex comprising main pilaster and accessory pilaster. Main pilaster consisting of large proximal glandular roll, distally lamellate and smooth robust distal muscular pleat, ending in genital atrium, branching or otherwise, near gonopore. Accessory pilaster slender, bridge-like, arising on one side of glandular roll and ending near gonopore; remarkably, medial portion detached from genital walls as free cordon. Vas deferens opening in transverse slit on glandular roll at about half its length. Internal surface smooth.

Genital Atrium (Figs. 39, 40): long and cylindrical, with one side connected to body wall by robust muscular bands. Internal structures consisting of distal portion of main and accessory pilasters. Internal surface smooth.

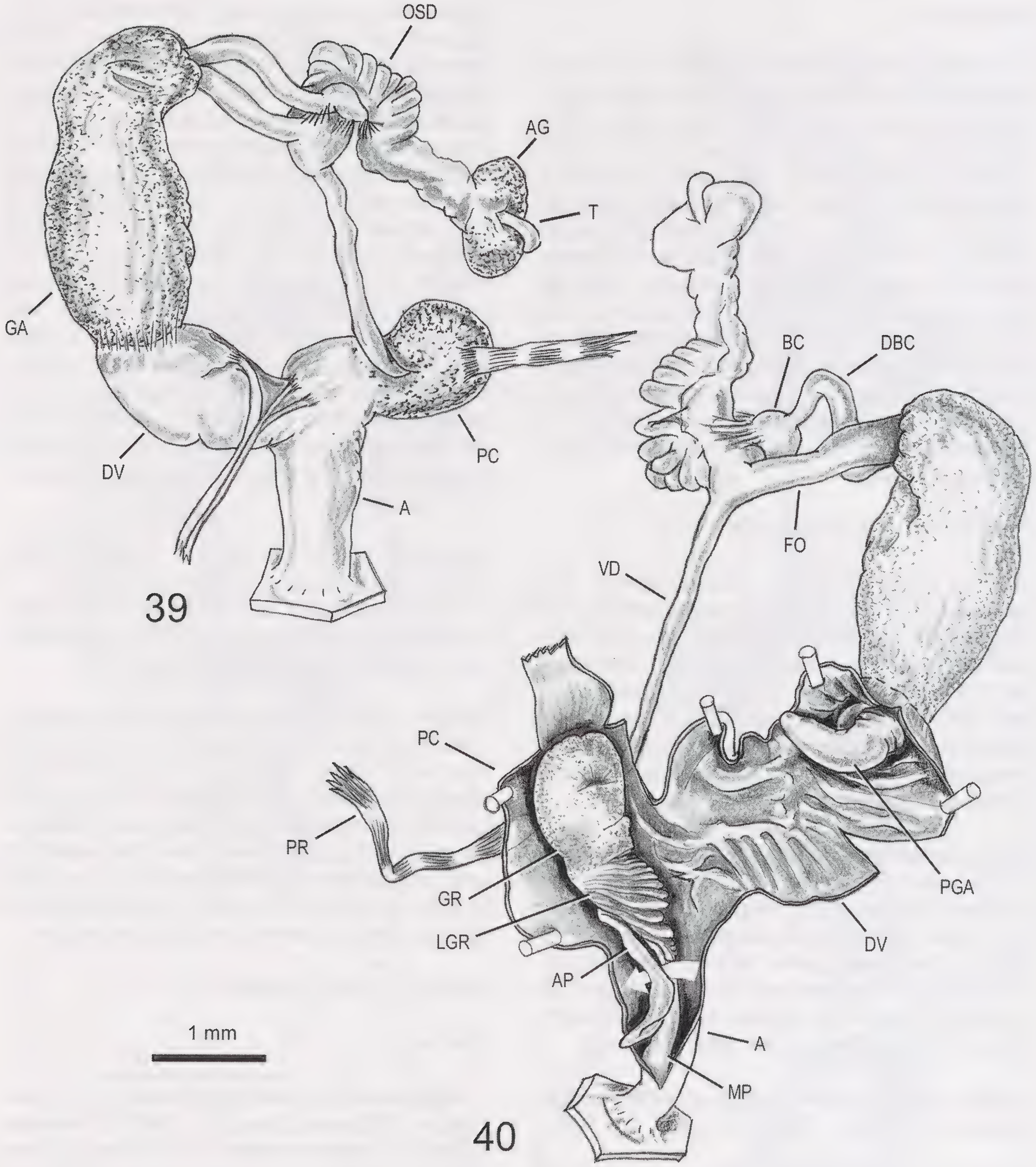
Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 3). Penial retractor long, passing between fork formed by retractors of right ommatophore and right lower tentacle, around right ocular nerve, then backwards to end on diaphragm; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.

Mating: no data available.

Remarks

Madeirovitrina was established as a subgenus of *Phenacolimax* by Groh & Hemmen (1986) for some species from Madeira. It was recently regarded as a subgenus of *Plutonia* by Alonso et al. (2000) and Hausdorf (2002). Hausdorf (2002) assigned six species to *Madeirovitrina*, two only tentatively (one was assigned to *Eucobresia* by Groh & Hemmen, 1986) (major anatomical references on *Madeirovitrina* species: Odhner, 1937; Groh & Hemmen, 1986).

The vaginal retractor muscle (Vaginaretraktormuskel) described by Groh & Hemmen (1986) probably corresponds to the genital nerve which originates from the right pedal ganglion and ends with numerous branches on

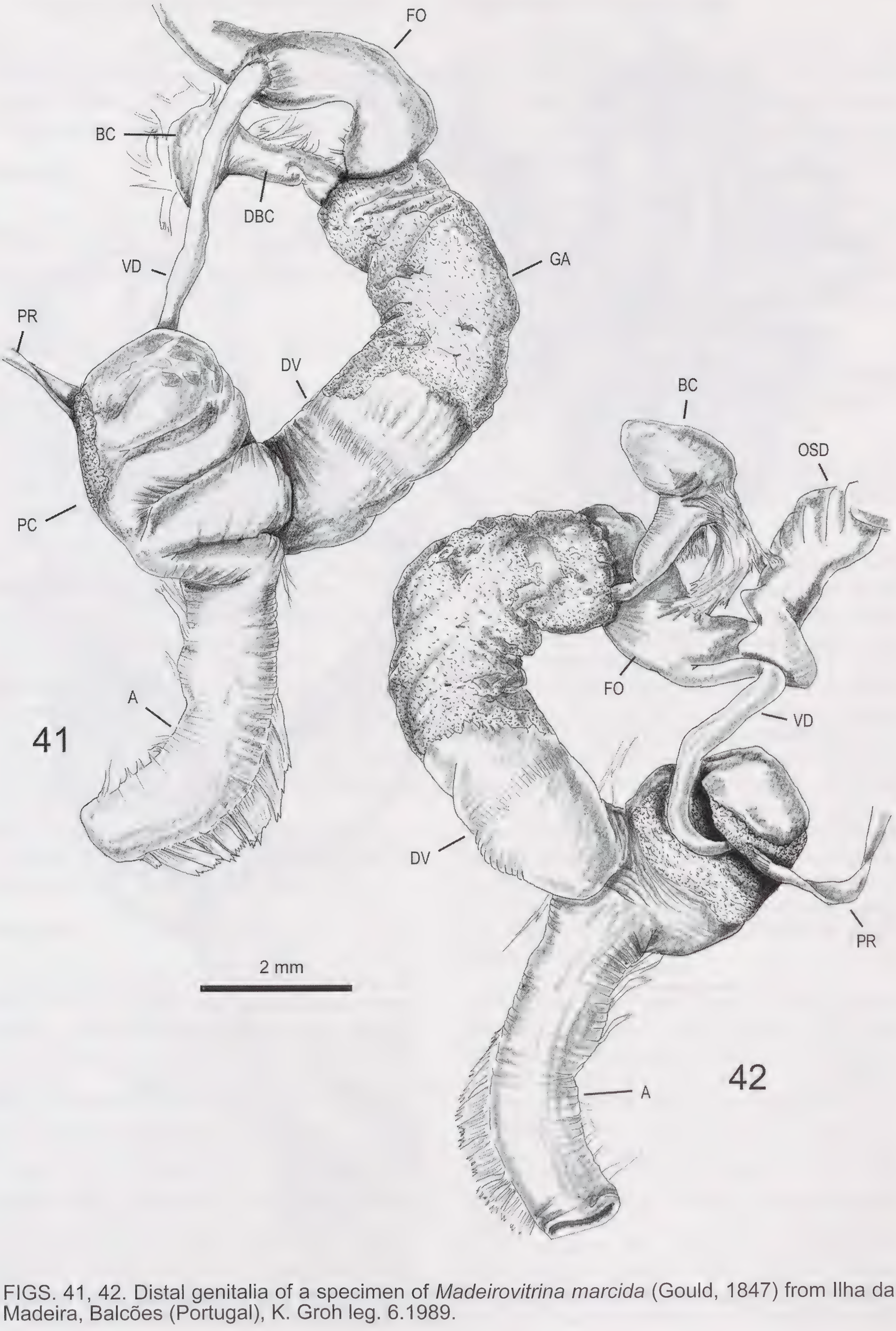


FIGS. 39, 40. *Madeirovitrina nitida* (Gould, 1847) from Ilha da Madeira, Pico Ruivo (Portugal), K. Groh leg. 7.1985. FIG. 39: Distal genitalia; FIG. 40: Internal structure of penial complex and vagina.

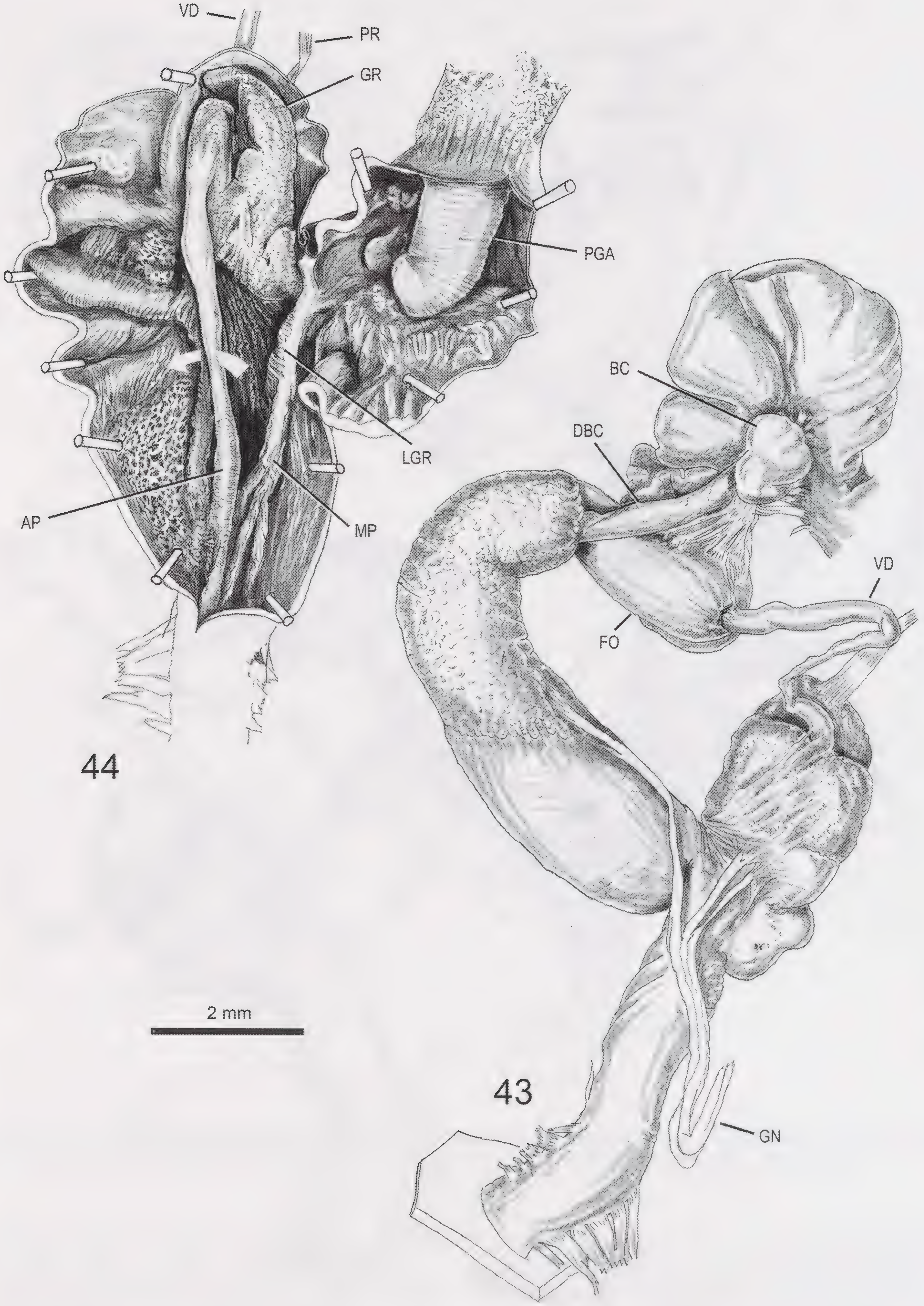
the distal penial complex and the vagina, near where the two enter the genital atrium.

The other *Madeirovitrina* species examined (*M. marcida*) differs from the type species in a number of characters (Figs. 41–44), but it shares a bridge-like accessory pilaster inside the penis.

All the analyses found that the two species of *Madeirovitrina* examined constitute a monophyletic group of Madeiran vitrinids supported by at least one exclusive synapomorphy, the bridge-like accessory pilaster (Figs. 14, 15; Table 3).



FIGS. 41, 42. Distal genitalia of a specimen of *Madeirovitrina marcida* (Gould, 1847) from Ilha da Madeira, Balcões (Portugal), K. Groh leg. 6.1989.



FIGS. 43, 44. *Madeirovitrina marcida* (Gould, 1847) from Ilha da Madeira, Balcões (Portugal), K. Groh leg. 6.1989. FIG. 43: Distal genitalia; FIG. 44: Internal structure of penial complex and vagina.

Oligolimax Fischer in: Paulucci, 1878

Type Species: *Vitrina (Oligolimax) paulucciae* Fischer in: Paulucci, 1878, by original designation. *Vitrina paulucciae* is a junior synonym of *Vitrina musignani* Pirajno, 1842.

Synonym

Gallandia Bourguignat, 1880. Type Species: *Vitrina conoidea* Martens, 1874, *sensu* Bourguignat, 1880, i.e., *G. olympica* Hausdorf, 1995.

Material Examined

Oligolimax annularis (Studer, 1820) – Italy: Alpi Apuane, Monte Pisanino, Valle Orto di Donna (Minucciano, Lucca), 32TNP9587, F. Giusti leg. 21.7.1970 (8 spirit specimens, FGC); Monte Terminillo (Rieti), 33TUH30, F. Giusti leg. 8.8.1966 (2 spirit specimens, FGC); Monti Sibillini, Val Canatra (Norcia, Perugia), 33TUH54, F. Giusti & G. Manganelli leg. 13.9.1988 (7 spirit specimens, FGC); Monte Pollino, Colle del Dragone (Morano Calabro, Cosenza), 33SWE9517, F. Giusti leg. 13.10.1977 (1 spirit specimen, FGC).

Oligolimax musignani (Pirajno, 1842) – Italy: Monti Nebrodi, Monte Soro, 1847 m (Cesarò, Messina), 33SVB7398, F. Giusti leg. 14.9.1981 (3 spirit specimens, FGC), G.B. Osella leg. 24.10.1981 (1 spirit specimen, FGC).

Oligolimax olympicus (Hausdorf, 1995) – Turkey: Orhangazi (Bursa), 35TPE98, B. Hausdorf leg. 25.9.1987 (1 shell, FGC).

Diagnosis

Snail-like vitrinids characterized by long penial retractor muscle arising from diaphragm, passing above right ommatophore retractor and ending on penial complex subapically; stimulator consisting of glandula amatoria with incomplete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens contacting penial complex about medially, running inside its wall and entering it subapically; internal structures of penial complex comprising main and accessory pilaster; main pilaster consisting of large, medially pleated, proximal glandular roll and thin distal muscular pleat.

Description of Type Species

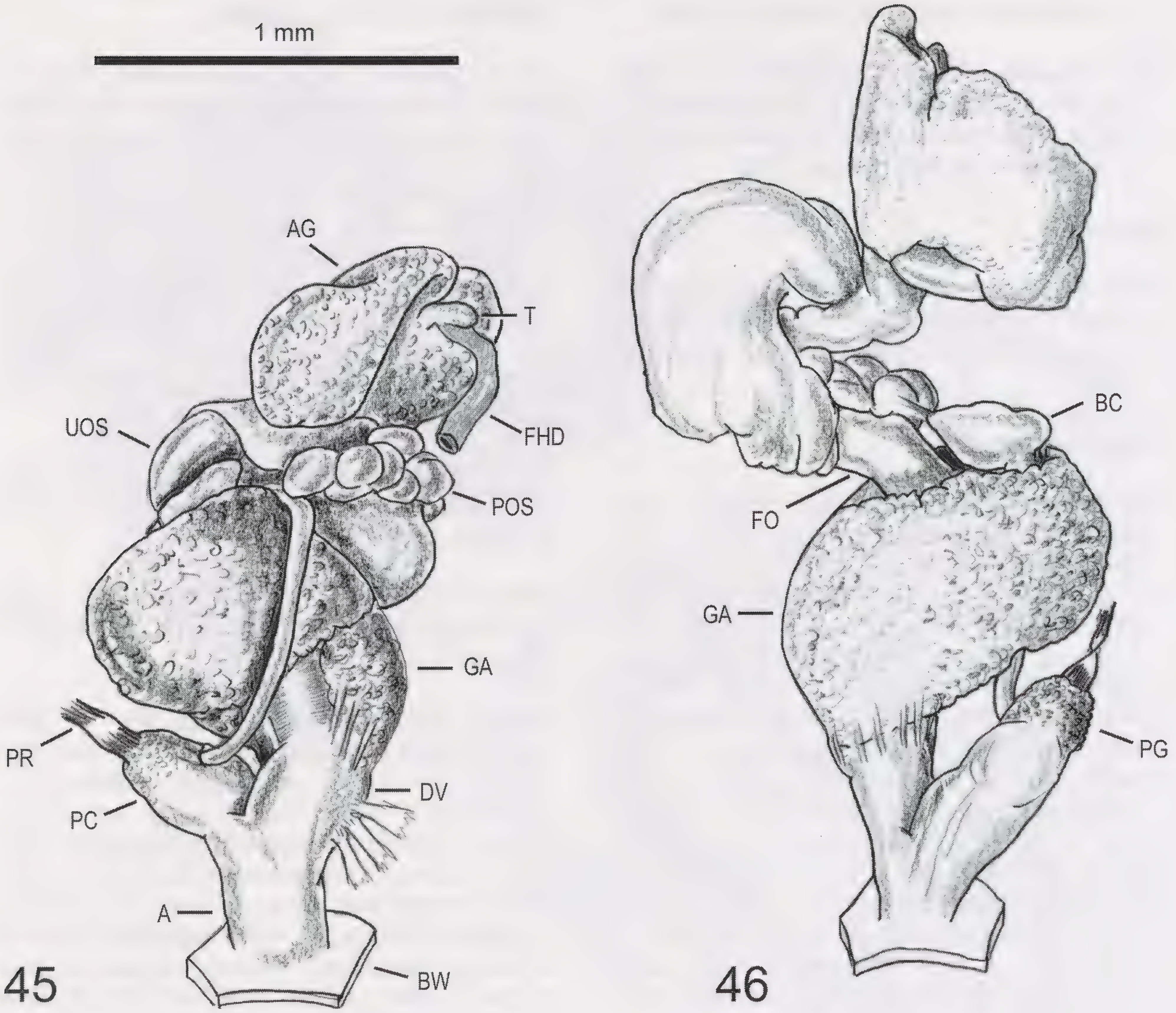
Shell: vitriniform typical, translucent, thin and fragile, globular-conical, sometimes angled with approximately 3.0 whorls; last whorl expanded; aperture moderately wide with columellar and basal margins bordered by narrow periostracal fringe; columellar margin normal; umbilicus open, punctiform; protoconch slightly prominent, with fine, wavy, irregularly branched, closely spaced, radial periostracal crests and isolated pits near suture; teleoconch with fine, closely and regularly spaced, slightly wavy, radial riblets (Manganelli & Giusti, 2004: figs. 1–7).

Body: can be withdrawn more or less completely into shell; right shell lobe absent (Manganelli & Giusti, 2004: fig. 9).

Radula: dichoglossan; marginal teeth partly pluricuspid (Manganelli & Giusti, 2004: 298–299, fig. 17).

Female Distal Genitalia (Figs. 45–48): free oviduct short and wide. Bursa copulatrix small and pyriform, with very short, slender duct, initially not flared. Vagina long, with large bulbous glandula amatoria constituting 1/2 to 2/3 of its length. Glandula amatoria with rather thin muscular wall, almost completely covered by external glandular layer; papilla of glandula amatoria very short. Internal surface of distal vagina rather smooth, with two longitudinal pilasters starting level with tip of papilla of glandula amatoria; larger pilaster rather robust, sometimes flanked by very thin pleats, originating from knob and ending by fusing with penial accessory pilaster; smaller pilaster very slender, ending by fusing with distal end of penial glandular roll.

Male Distal Genitalia (Figs. 45–48): penial complex very small and club-like, with penial gland and without penial sheath. Penial gland covering most of proximal penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens medium in length, contacting penial complex about medially, then running inside thickness of penial gland and entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex comprising main and accessory pilaster. Main pilaster consisting of large proximal glandular roll, divided into



FIGS. 45, 46. Distal genitalia of a specimen of *Oligolimax musignani* (Pirajno, 1842) from Monti Nebrodi, Monte Soro, 1847 m (Sicily, Italy), G. B. Osella leg. 24.10.1981.

initial yellow knoll, pale yellow medial portion and transversely pleated final portion (small dimensions prevent establishing homology with distal lamellate portion of glandular roll of other taxa) and thin distal muscular pleat fusing with lesser vaginal pleat. Accessory pilaster slender, arising on one side of glandular roll and ending by fusing on one side with pleat or system of pleats of genital atrium and, on other, with larger vaginal pleat. Vas deferens opening at top of distinct yellow knoll constituting initial part of glandular roll. Internal surface smooth.

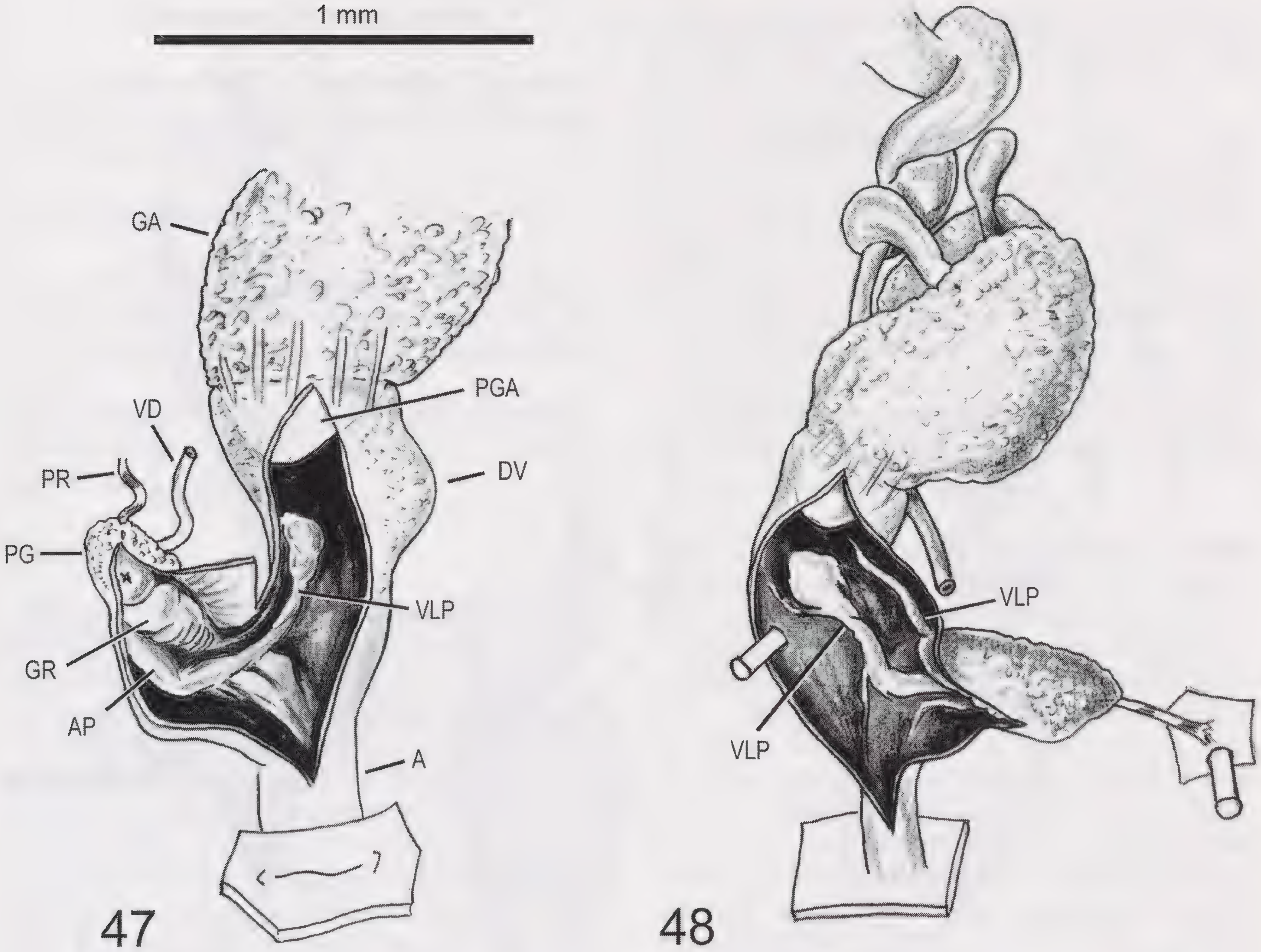
Genital Atrium (Figs. 45–48): short, with one side connected to body wall by robust muscular bands. Internal structures consisting of longitudinal pleat or system of pleats. Internal surface smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 9). Penial retractor long, passing above right ommatophore retractor which is consequently free of penial complex and vagina.

Mating: no data available.

Remarks

Oligolimax was described by P. Fischer (in: Paulucci, 1878) as subgenus of *Vitrina* with two species originally included: *Vitrina paulucciae* Fischer in: Paulucci, 1878, and *Vitrina annularis* (Studer, 1820). It was subsequently regarded as a subgenus of *Phenacolimax* by Hesse (1923), Forcart (1957, 1959) and Zilch (1959), a section



FIGS. 47, 48. *Oligolimax musignani* (Pirajno, 1842) from Monti Nebrodi, Monte Soro, 1847 m (Sicily, Italy), F. Giusti leg. 14.9.1981. FIG. 47: Distal genitalia; FIG. 48: Internal structure of penial complex and vagina.

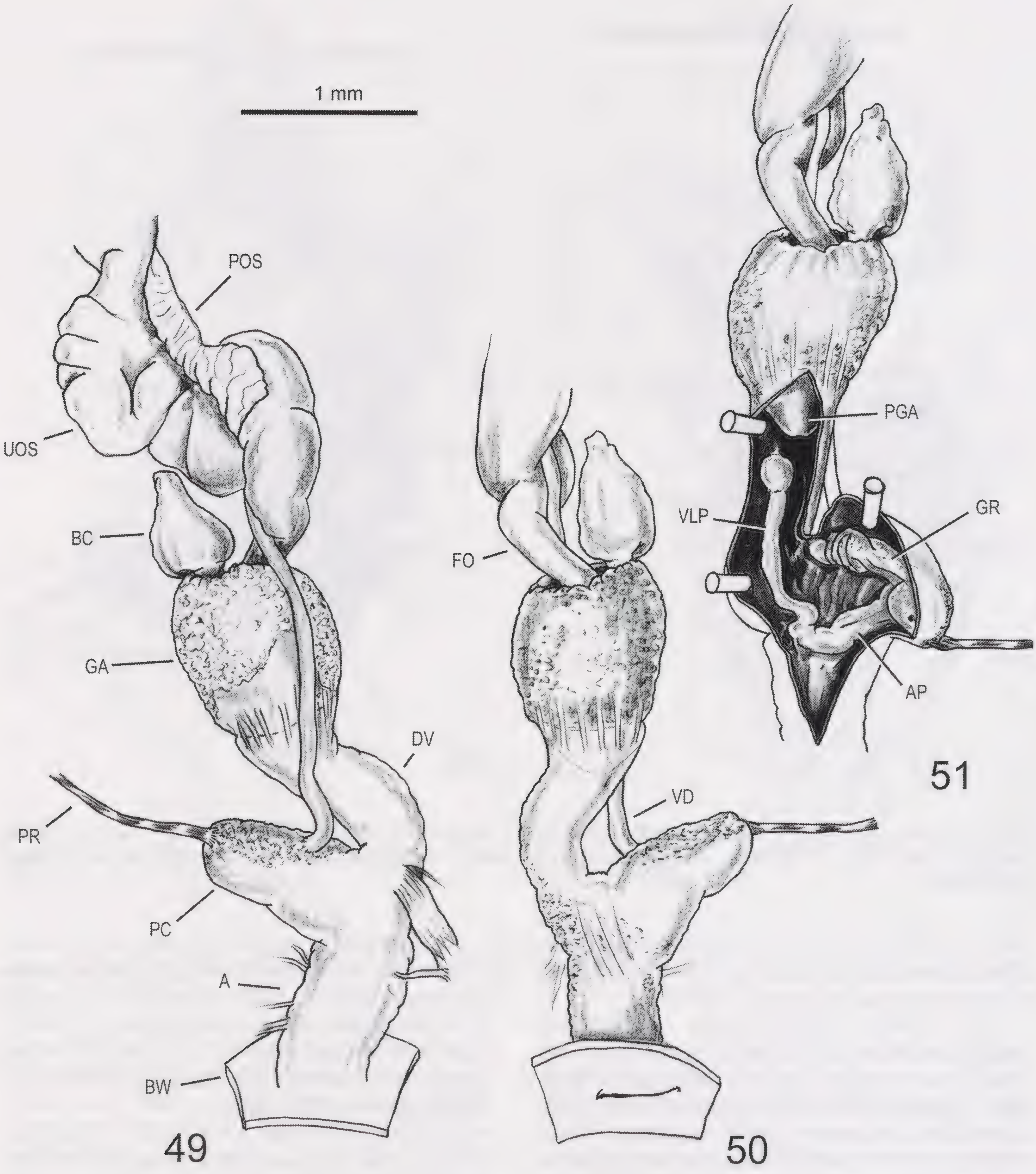
of subgenus *Vitrina* (s.s.) by Thiele (1931) and a distinct genus by Forcart (1944).

In the 1960s, Forcart (1965) claimed that the type-species was a junior synonym of *Vitrina bonelli* Targioni Tozzetti, 1873, and hence that *Oligolimax* had been used as a name for a completely different group of species, those hitherto included in *Semilimacella*, whereas the species hitherto included in *Oligolimax* had to be assigned to *Gallandia* Bourguignat, 1880 (type species: *Vitrina conoidea* Martens, 1874, *sensu* Bourguignat, 1880, i.e., *G. olympica* Hausdorf, 1995). *Gallandia* was subsequently regarded as a junior synonym of *Phenacolimax* by Schileyko (1986) and a distinct genus by Hausdorf (1995), Neubert (1998) and Alonso et al. (2000).

In a checklist of Italian malacofauna, Manganelli et al. (1995) reported that study of the syntypes of *V. paulucciae* demonstrated that Forcart (1965) was wrong to consider this species a junior synonym of *V. bonelli* and that *Oligolimax* was indeed available in the sense of Hesse (1924). Subsequently, Manganelli & Giusti (2004) demonstrated that *V. paulucciae* was in turn a junior synonym of *Vitrina musignani* Pirajno, 1842, and gave a complete redescription of the latter.

According to Hausdorf (2002), *Oligolimax* includes six species from the western Palaearctic (major anatomical references on which are: Forcart, 1959; Schileyko, 1986; Hausdorf, 1995, 2002; Manganelli & Giusti, 2004) and is the sister group of *Plutonia*; in turn, *Plutonia* and *Oligolimax* are the sister group of *Arabivitrina* and *Calidivitrina*.

Two out of the six species assigned to *Oligolimax* by Hausdorf (2002) belong to other genera (*O. lederi* to *Trochovitrina*; *O. pollonerianus* to *Sardovitrina*). Apart from *O. sturanyi*, the other species are well known anatomically (Hausdorf, 1995; Manganelli & Giusti, 2004). They have distal genitalia very similar in general scheme



FIGS. 49–51. *Oligolimax annularis* (Studer, 1820) from Monti Sibillini, Val Canatra (Umbria, Italy), F. Giusti & G. Manganelli leg. 13.9.1988. FIGS. 49, 50: Distal genitalia; FIG. 51: Internal structure of penial complex and vagina.

and internal structure, differing only in minor details (length of papilla of glandula amatoria, length of vagina, extension of glandular covering of glandula amatoria; see Manganelli & Giusti, 2004). Various nominal taxa of uncertain status were also assigned to this genus (cf. Manganelli & Giusti, 2004).

Most analyses find *Oligolimax* to be monophyletic, supported by non exclusive synapomorphies (Figs. 14, 15; Table 3); *Oligolimax* in turn constitutes a monophyletic group along with *Sardovitrina*, *Semilimacella* and *Vitrina*, based on the same relationships between the right ommatophore retractor and the distal genitalia.

Phenacolimax Stabile, 1859

Type Species: *Helicolimax major* Férussac, 1807, by subsequent designation (Fischer in: Paulucci, 1878).

Material Examined

Phenacolimax blanci (Pollonera, 1884) – Italy: Grotta della Melosa (Pigna, Imperia), G. Comotti leg. 2.8.1982 (4 spirit specimens, FGC).

Phenacolimax major (Férussac, 1807) – France: Mialet (Gard), M. Bodon leg. 1.12.1984 (2 spirit specimens, FGC).

Diagnosis

Semislug-like vitrinids characterized by short penial retractor muscle arising from diaphragm, compelling penial complex below right ommatophore and in front of right optic nerve and ending on penial complex subapically (but juveniles with long penial retractor muscle running in front of right optic nerve, below right ommatophore retractor); stimulator consisting of glandula amatoria with incomplete external glandular covering; penial complex with penial gland and penial sheath; vas deferens contacting penial complex medially, traversing penial sheath, running inside penial wall and entering it subapically; internal structures of penial complex comprising main pilaster, two accessory pilasters and tongue-like structure; main pilaster consisting of large, not clearly distally lamellate, proximal glandular roll and wrinkled distal muscular pleat.

Description of Type Species

Shell: vitriniform typical, glossy, thin and fragile, depressed, with approximately 2.5–3.0 whorls; last whorl expanded; aperture moderately wide with columellar and basal margins bordered by narrow periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 7, fig. 4a–c).

Body: cannot be withdrawn completely into shell; right shell lobe medium, covering limited portion of shell when animal undisturbed (Kerney & Cameron, 1979: pl. 7, fig. 4d; Falkner, 1992: figs. 11, 12 at p. 271).

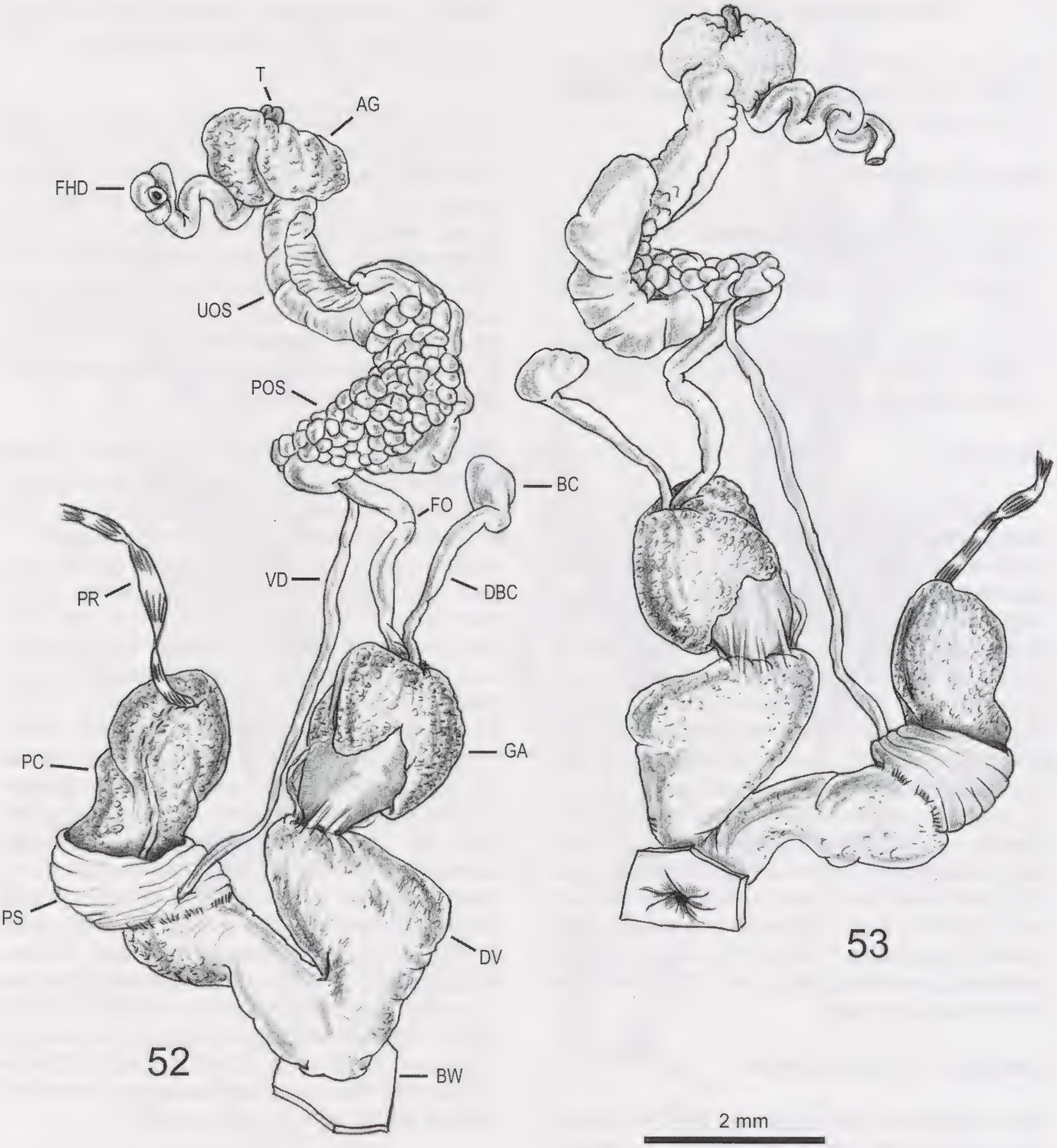
Radula: dichoglossan; marginal teeth bicuspid (with one small ectocone) (Hesse, 1923: 99–100, pl. 1, fig. 7).

Female Distal Genitalia (Figs. 52, 53, 55, 56): free oviduct long and slender. Bursa copulatrix small and oval to shoe-shaped, with short to medium (?), very slender duct, initially not flared. Vagina long, with small heart-like glandula amatoria constituting half its length. Glandula amatoria with very thick muscular wall, covered only proximally by external glandular layer; papilla of glandula amatoria very short. Internal surface of distal vagina with many thin, regular, transverse pleats.

Male Distal Genitalia (Figs. 52–54): penial complex large and club-like, with penial gland and penial sheath. Penial gland covering most of penial complex. Penial sheath medium in length, curled up, arising from mid penial complex and enveloping most of proximal penial complex. Penial retractor arising from diaphragm, short, inserted apically. Vas deferens long, contacting penial complex about medially, then traversing penial sheath, running inside thickness of penial gland and entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex comprising main pilaster, two accessory pilasters and tongue-like structure. Main pilaster consisting of large proximal glandular roll, not clearly distally lamellate, and wrinkled distal muscular pleat ending in genital atrium side by side with two accessory pilasters. Accessory pilasters arising near tongue-like structure opposite glandular roll and ending in genital atrium. Vas deferens opening in transverse slit on glandular roll at about half its length. Internal surface with low irregularly arranged pleats and sparse spongy tissue with crypts.

Genital Atrium (Figs. 52–55): very short, without muscular bands. Internal structures consisting of distal portion of main and accessory pilasters. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: age-dependent (Figs. 3, 6). Juveniles with long slender penial retractor passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina. Adults with penial retractor short so



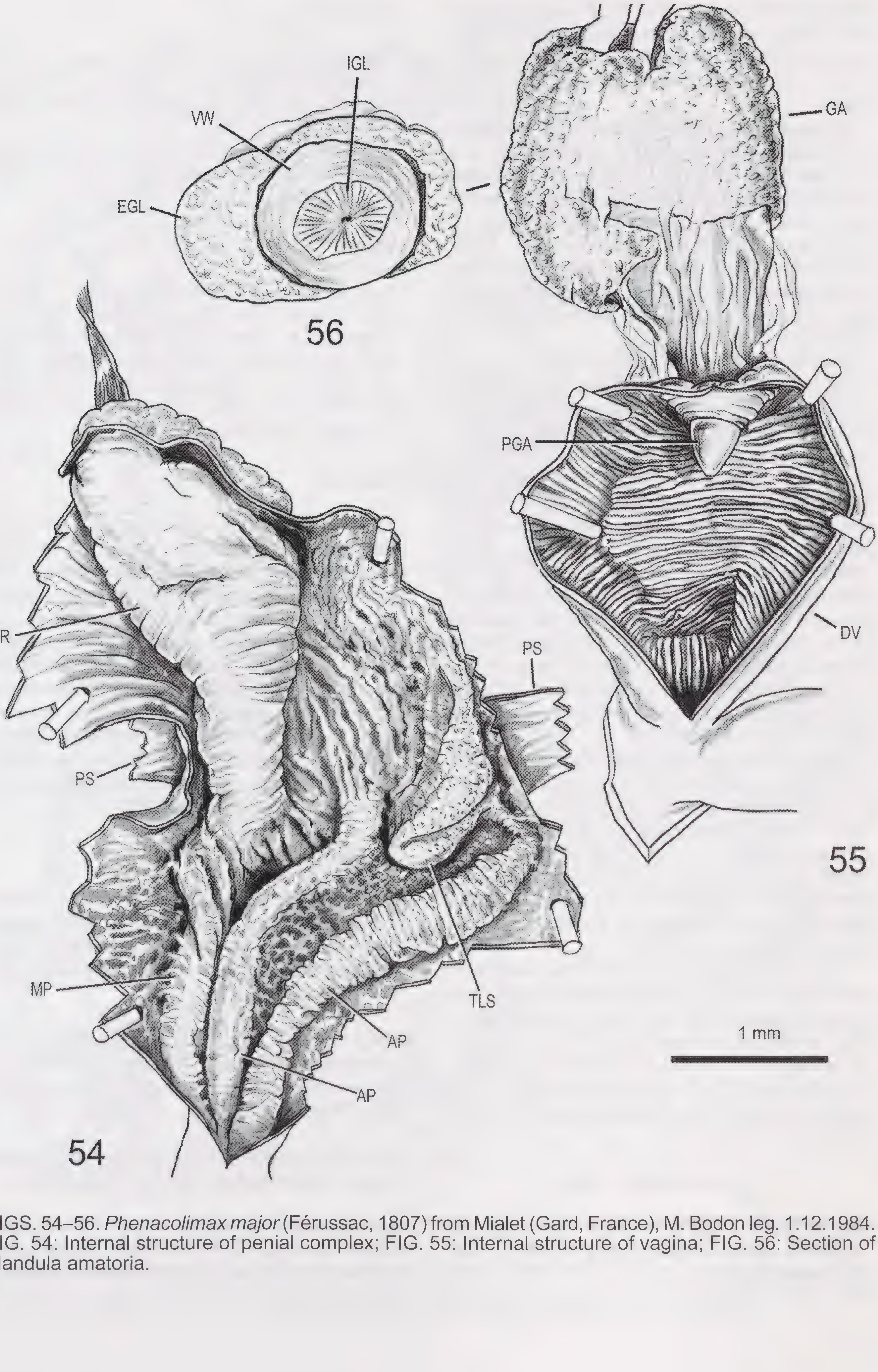
FIGS. 52, 53. Distal genitalia of a specimen of *Phenacolimax major* (Férussac, 1807) from Mialet (Gard, France), M. Bodon leg. 1.12.1984.

that entire penial complex is below right ommatophore and in front of right optic nerve; right ommatophore is consequently shifted right and compelled to lie between penial complex and vagina.

Mating: Hausdorf (2002) stated that stimulator is not used for fixing partner and penis is not inserted into vagina or duct of bursa copulatrix during copulation.

Remarks

Phenacolimax was established by Stabile (1859) with four species originally included. Overlooking the designation of *Helicolimax major* Férussac, 1807, as type species, Hesse (1923) considered *Phenacolimax* to belong to the "Gruppe der *V. pellucida* und *diaphana*". A few years later, Baker (1929) corrected Hesse's misinterpretation, restoring the correct



FIGS. 54–56. *Phenacolimax major* (Férussac, 1807) from Mialet (Gard, France), M. Bodon leg. 1.12.1984. FIG. 54: Internal structure of penial complex; FIG. 55: Internal structure of vagina; FIG. 56: Section of glandula amatoria.

sense of this taxon. *Phenacolimax* has since then been considered a distinct genus by all authors except Thiele (1931) and Hubendick (1953) who regarded it as a subgenus of *Vitrina*.

Although many species have been assigned to this genus in the course of time, only four species from western Europe are attributed now to *Phenacolimax* (Hausdorf, 2002; Gavetti et al., 2008) (major anatomical references on which are: Forcart, 1949; Boato et al., 1986; De Winter, 1990; Neubert, 1998). According to Hausdorf (2002), *Phenacolimax* is the sister group of ((*Arabivitrina*, *Calidivitrina*) (*Plutonia*, *Oligolimax*)).

Moquin Tandon (1855, 2: 49–52, Atlas: pl. 6, figs. 14–32) gave a detailed but incorrect description of the male distal genitalia of *P. major*. According to him, the penial complex had a very large, long proximal part bent on itself and referred to as "flagellum épais". Actually, Moquin Tandon was unable to see that the vas deferens only contacted the penis at about half of the penis length, traversed the penial sheath, ran inside the penial gland and ended near the penis apex. Moquin Tandon's description and figure probably inspired the scheme of the distal genitalia of *P. major* published by Germain (1930: fig. 89), since it reproduces a penial complex with vas deferens entering penis at half its length and with a long flagellum twisted in the shape of an "S".

Phenacolimax species differ by virtue of genital atrium length (short vs. long), point of origin of penial sheath (distal vs. medial) and other minor details. *Phenacolimax* shares the same age-dependent relationships between right ommatophore retractor and distal genitalia with *Arabivitrina*, but is distinct by virtue of a penial sheath and the absence of a distal lamellate portion of the glandular roll.

There is no evidence that *Phenacolimax* species constitute a monophyletic group. Suspecting a relationship between *Eucobresia* and *Phenacolimax*, we investigated this hypothesis by phylogenetic analysis of three species of *Eucobresia* and two of *Phenacolimax*, but no clade including these species, or at least some of them, was recovered.

Plutonia Stabile, 1864

New name for *Viquesnelia* Morelet, 1860 (type species *Viquesnelia atlantica* Morelet, 1860, by monotypy).

Synonyms

Viquesnelia Morelet, 1860. Type Species: *Viquesnelia atlantica* Morelet, 1860, by monotypy. *Non Viquesnelia* Deshayes, 1857

Vitriplutonia Collinge, 1893, new name for *Plutonia* Stabile, 1864 (according to Bank et al., 2002).

Material Examined

Plutonia atlantica (Morelet, 1860) – Portugal: Ilha de São Miguel (Azores), Serra de Tronqueira, T. Backeljau leg. 28.6.1989 and 6.6.1990 (5 spirit specimens, IRSNB); Pico do Carvão, T. Backeljau leg. 16.7.1989, 8.6.1990, 26.6.1989, 16.7.1989 and 3.7.1989 (22 spirit specimens, IRSNB).

Diagnosis

Slug-like vitrinids characterized by short modified penial retractor (consisting of three branches, none reaching penial complex: first reaches bursa copulatrix and its duct, second reaches free oviduct and third reaches vas deferens); stimulator consisting of glandula amatoria with complete external glandular covering; penial complex with penial gland and without penial sheath; vas deferens entering penial complex apically; internal structures of penial complex comprising main and accessory pilasters (?); main pilaster comprising large, not distally lamellate glandular roll.

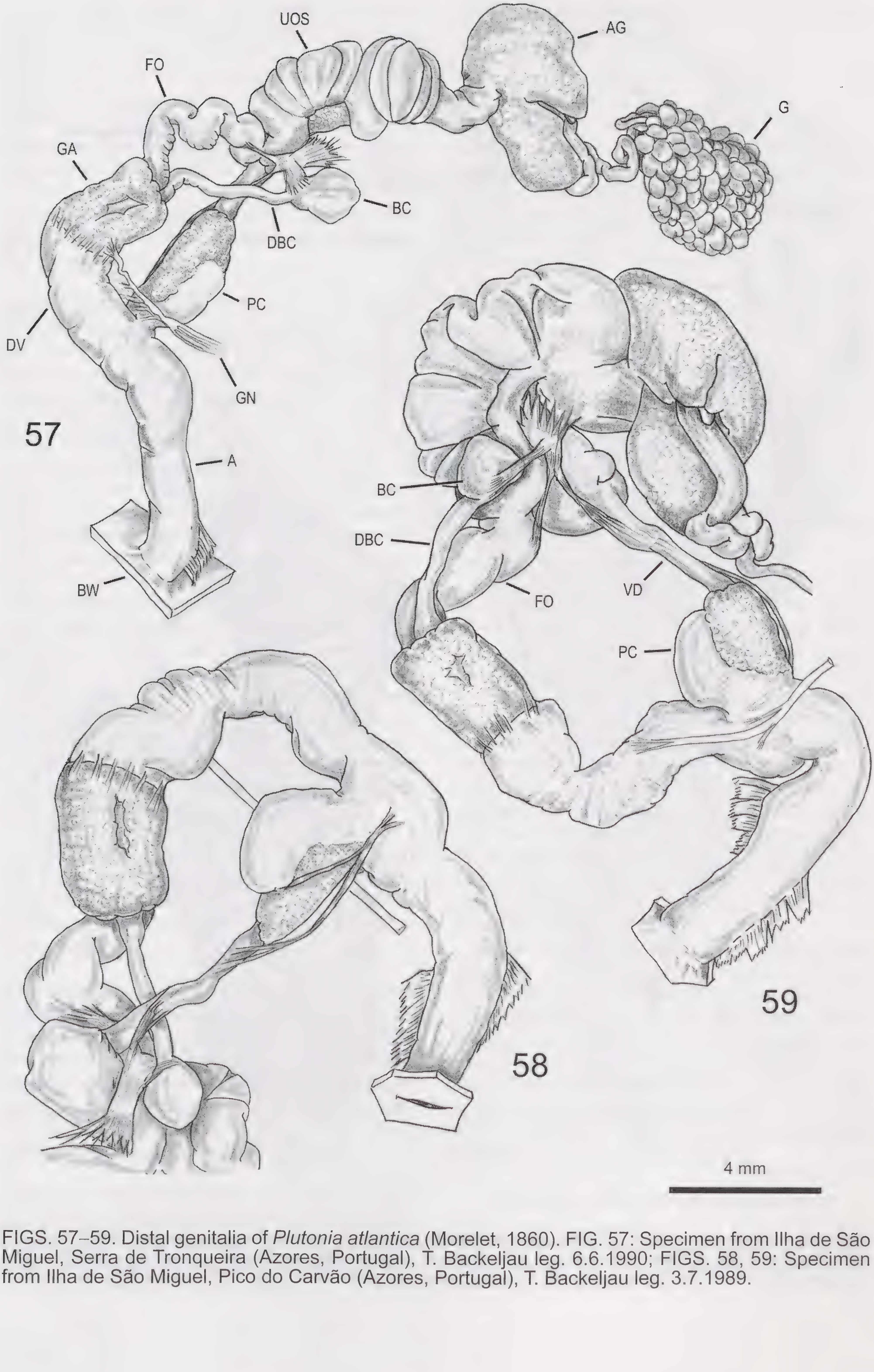
Description of Type Species

Shell: internal, limacella-like, rather thin, convex and with traces of growth lines above, slightly concave below, with nucleus (protoconch area) lying over sort of basal tab (Wiktor & Backeljau, 1995: figs. 5–10).

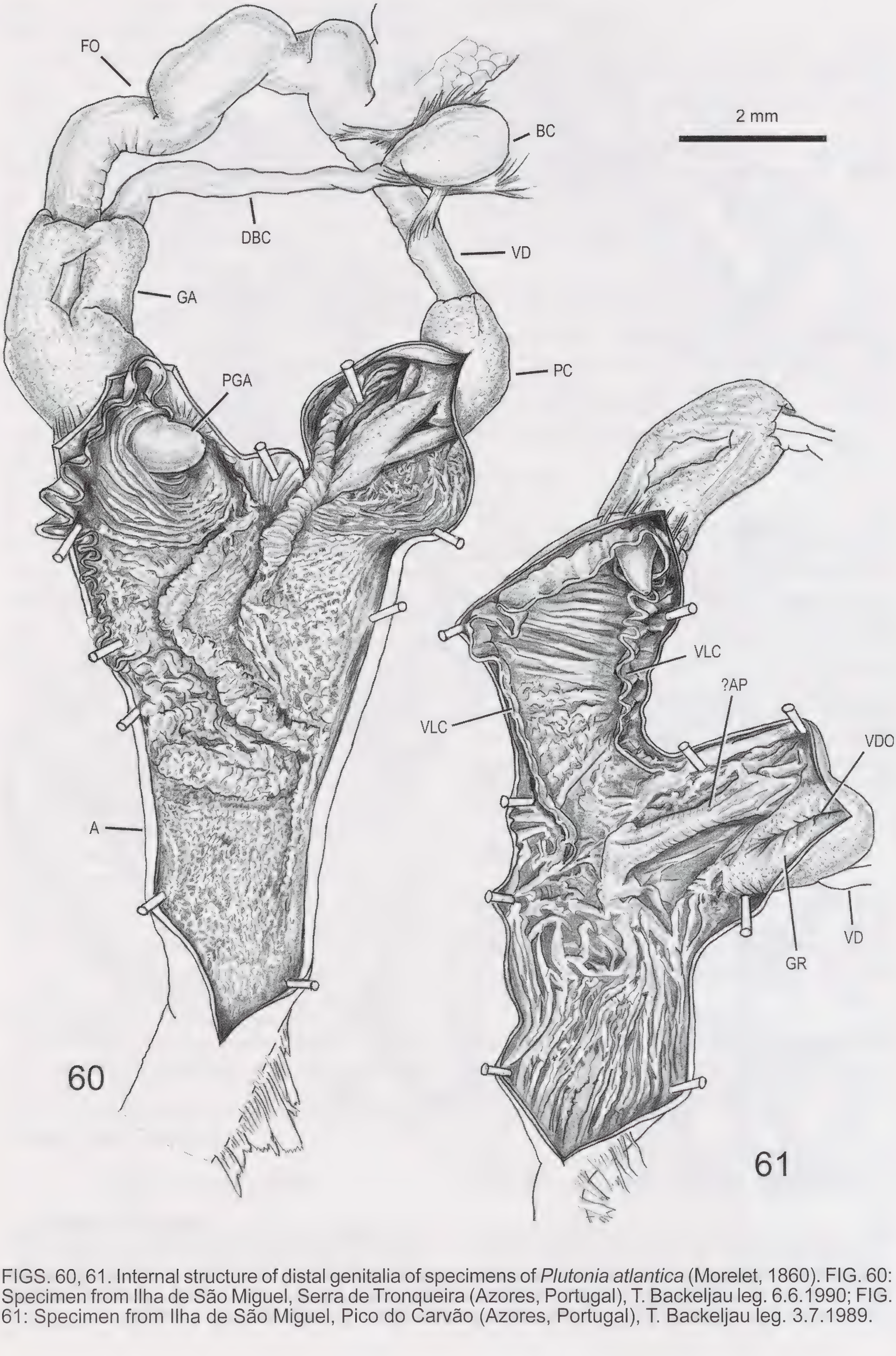
Body: slug-like having large oval clypeus with postmedian pneumostome and tail laterally compressed with evident continuous dorsal keel (Wiktor & Backeljau, 1995: figs. 1–4).

Radula: beloglossan; all teeth monocuspid (Wiktor & Backeljau, 1995: 75, figs. 22–25).

Female Distal Genitalia (Figs. 57–61): free oviduct short to long and wide. Bursa copulatrix small and oval, with short to medium,



FIGS. 57–59. Distal genitalia of *Plutonia atlantica* (Morelet, 1860). FIG. 57: Specimen from Ilha de São Miguel, Serra de Tronqueira (Azores, Portugal), T. Backeljau leg. 6.6.1990; FIGS. 58, 59: Specimen from Ilha de São Miguel, Pico do Carvão (Azores, Portugal), T. Backeljau leg. 3.7.1989.



FIGS. 60, 61. Internal structure of distal genitalia of specimens of *Plutonia atlantica* (Morelet, 1860). FIG. 60: Specimen from Ilha de São Miguel, Serra de Tronqueira (Azores, Portugal), T. Backeljau leg. 6.6.1990; FIG. 61: Specimen from Ilha de São Miguel, Pico do Carvão (Azores, Portugal), T. Backeljau leg. 3.7.1989.

more or less slender duct, initially not flared. Vagina long, with small cylindrical glandula amatoria constituting 1/2 to 2/3 of its length. Glandula amatoria with thick muscular wall, almost completely covered by external glandular layer; papilla of glandula amatoria short. Internal surface of distal vagina with thin, mainly transverse pleats and two longitudinal wavy laminar crests (one larger and longer and the other sometimes less evident) starting from base of papilla of glandula amatoria and ending before genital atrium.

Male Distal Genitalia (Figs. 57–61): penial complex small and nearly bilobate, with penial gland and without penial sheath. Penial gland covering one side of penial complex. Penial retractor arising from diaphragm, short modified and divided into three branches, none of which reaches penial complex: first reaches bursa copulatrix and its duct, second free oviduct and third vas deferens (sometimes thin muscular branches connect distal vas deferens and penial complex). Vas deferens short and thick, entering penial complex apically. Internal structures of penial complex comprising main and accessory pilasters. Main pilaster consisting of large glandular roll, not distally lamellate. Accessory pilaster more or less superficially pleated, arising near proximal glandular roll and ending in proximal genital atrium. Vas deferens opening inside long longitudinal groove on glandular roll (in an adult specimen examined, sort of small tongue-like structure projected over groove of glandular roll). Internal surface with low, irregularly arranged pleats.

Genital Atrium (Figs. 57–61): very long and cylindrical, with opposite sides connected to body wall by robust muscular bands. Internal surface with thin mainly longitudinal pleats or spongy tissue.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: due to the fact that penial retractor is modified and divided into three branches, none of which reaches penial complex, right ommatophore retractor is free of distal genitalia (in juveniles of a single population right ommatophore retractor was found both below and above penial complex).

Mating: no data available. Hausdorf (2002) stated that in *Plutonia* penis is not inserted into vagina or duct of bursa copulatrix during copulation. However this observation is not based on *P. atlantica* but on some other species he included in this genus.

Remarks

Plutonia was introduced by Stabile (1864) as a new name for *Viquesnelia* Morelet, 1860, a genus established for a slug-like vitrinid of the Azores, *Viquesnelia atlantica*, which turned out to be a junior homonym of *Viquesnelia* Deshayes, 1857.

Simroth (1891) claimed that *Plutonia* was related to other Macaronesian vitrinids and this was accepted by all subsequent authors, except Wiktor & Backeljau (1995), who supported its inclusion in a distinct family (Plutoniidae). *Plutonia* has been regarded as a distinct monospecific genus by Simroth (1891), Thiele (1931) and Schileyko (1986), a subgenus of *Phenacolimax* by Forcart (1957), Zilch (1959) and Backhuys (1975), a genus divided into five subgenera including all Macaronesian vitrinds by Alonso et al. (2000) and Hausdorf (2002) and a genus divided into two subgenera (*Plutonia* s.s. and *Canarivitrina*) by Schileyko (2003).

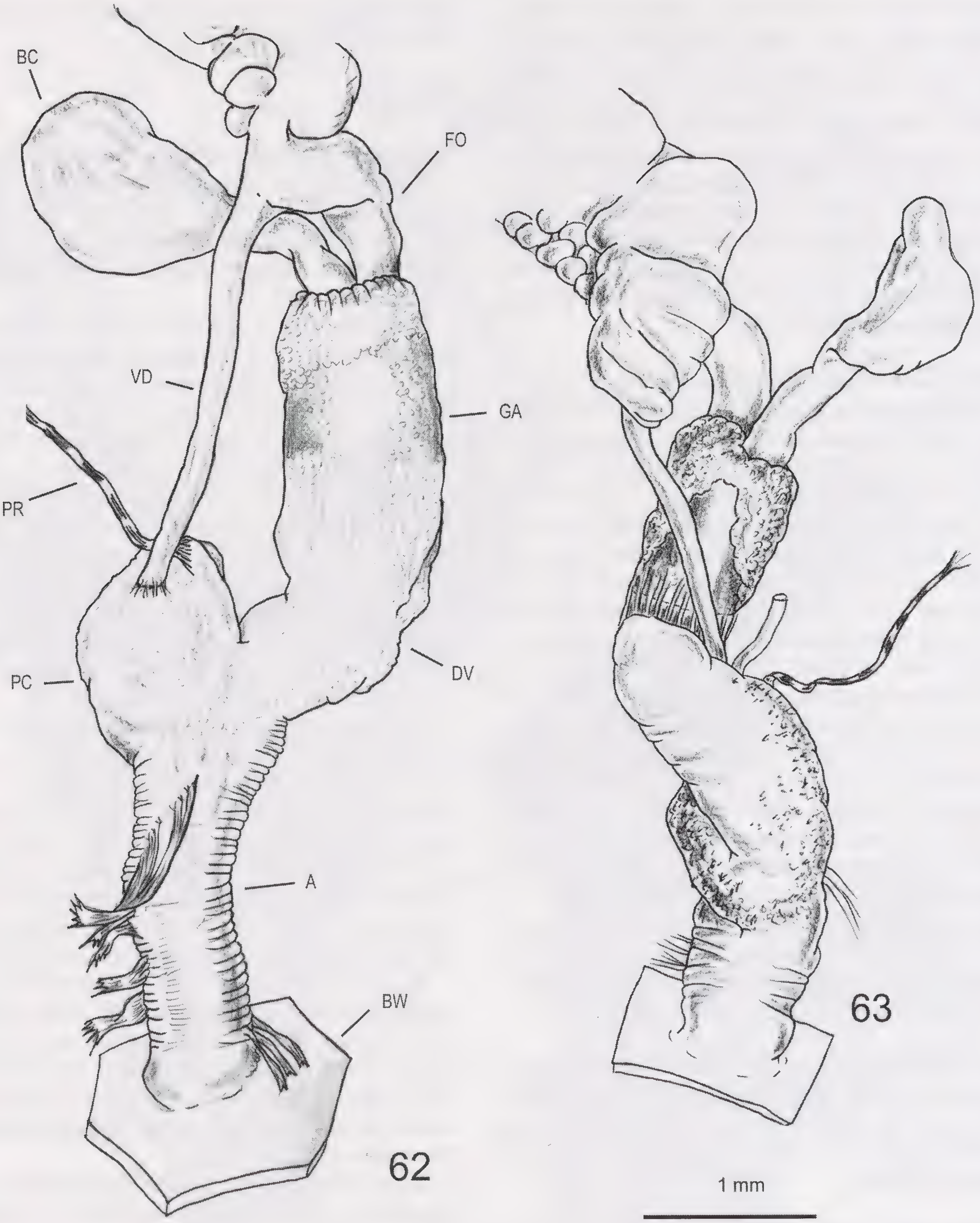
Plutonia includes only the type species, *P. atlantica* (major anatomical references on which are: Simroth, 1891; Wiktor & Backeljau, 1995) although it may constitute a group of cryptic species (Medeiros et al., 2000). According to Hausdorf (2002), *Plutonia* (as including all Macaronesian vitrinds) is the sister group of *Oligolimax*; in turn *Plutonia* and *Oligolimax* are the sister group of *Arabivitrina* and *Calidivitrina*.

Study of many specimens from different localities showed remarkable difference in shape and internal ornamentation of the various parts of the distal genitalia (compare Figs. 57–61). This might suggest the existence of more than one species of *Plutonia*, as hypothesized by Medeiros et al. (2000).

Some other Azorean semislug vitrinids, assigned to *Azorivitrina* (*A. brumalis*, *A. finitima* and *A. laxata*), share the same scheme of the distal genitalia (long to very long free oviduct, vagina and genital atrium; short to very short penial complex) with *Plutonia atlantica* (cf. Figs. 19–22; see also Mordan & Martins, 2001). However, phylogenetic analysis does not recover any monophyletic group including *Plutonia atlantica* and *Azorivitrina* species.

Sardovitrina Manganelli & Giusti, 2005

Type Species: *Vitrina polloneriana* [Arbanasich], 1897, by original designation.



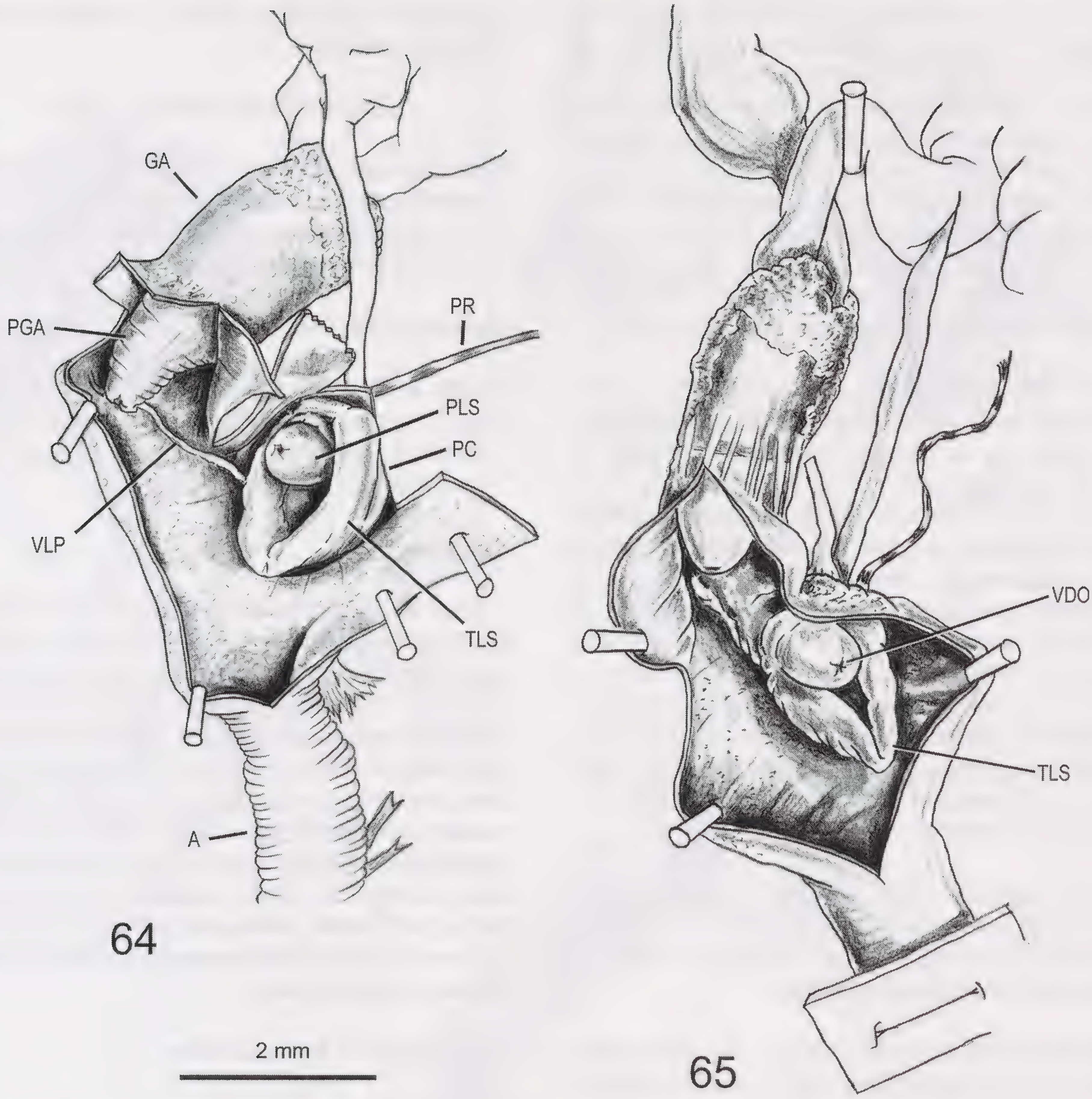
FIGS. 62, 63. Distal genitalia of specimens of *Sardovitrina polloneriana* ([Arbanasich], 1897). FIG. 62: Specimen from Lanusei (Sardinia, Italy), J. A. J. Nienhuis leg. 25.1.1972; FIG. 63: Specimen from Arbatax (Sardinia, Italy), J. A. J. Nienhuis leg.

Material Examined

Sardovitrina polloneriana ([Arbanasich], 1897) – Italy: Arbatax (Tortolì, Nuoro), 32SNK62, J. A. J. Nienhuis leg. (7 spirit specimens, FGC); Lanusei (Lanusei, Nuoro), 32TNK41, J. A. J. Nienhuis leg. 25.1.1972 (10 spirit specimens, FGC).

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, passing above right ommatophore retractor and ending on penial complex apically; stimulator consisting of glandula amatoria; penial complex very small with penial gland and without penial



FIGS. 64, 65. Internal structure of penial complex and vagina of specimens of *Sardovitrina polloneriana* ([Arbanasich], 1897). FIG. 64: Specimen from Lanusei (Sardinia, Italy), J. A. J. Nienhuis leg. 25.1.1972; FIG. 65: Specimen from Arbatax (Sardinia, Italy), J. A. J. Nienhuis leg.

sheath; vas deferens entering penial complex subapically; internal structures of penial complex distinctive and consequently difficult to homologize, comprising short, rolled, tongue-like structure embracing distinct, cylindrical, papilla-like structure.

Description of Type Species

Shell: vitriniform typical, glossy, thin and fragile, depressed, with approximately 3.0 whorls, last whorl expanded; aperture moderately wide with columellar and basal margins bordered by narrow periostracal fringe; columellar margin normal; umbilicus open, punctiform; protoconch slightly prominent with blunt, closely spaced, radial ribs and very small pits; teleoconch smooth (Manganelli & Giusti, 2005: figs. 1–6).

Body: cannot be completely withdrawn into shell; right shell lobe medium, covering limited portion of shell when animal undisturbed (Manganelli & Giusti, 2005: fig. 7).

Radula: dichoglossan; marginal teeth pluricuspid (Manganelli & Giusti, 2005: 574–575, figs. 16–18).

Female Distal Genitalia (Figs. 62–65): free oviduct short and wide. Bursa copulatrix medium in size and sac-like, with short, slender duct, initially not flared. Vagina long, with small cylindrical glandula amatoria constituting half its length. Glandula amatoria with very thick muscular wall, covered only proximally by external glandular layer; papilla of glandula amatoria short. Internal surface of distal vagina rather smooth, with spongy glandular tissue and one very thin pleat starting level with tip of papilla of glandula amatoria and ending by fusing with base of penial rolled tongue-like structure.

Male Distal Genitalia (Figs. 62–65): penial complex very small and globular (sometimes so short as to appear simple outgrowth of distal vagina), with penial gland and without penial sheath. Penial gland covering sparsely most of penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens medium in length, entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex distinctive and therefore difficult to homologize, comprising short, rolled tongue-like structure embracing distinct, cylindrical papilla-like structure. Vas deferens opening at tip of papilla-like structure. Internal surface smooth.

Genital Atrium (Figs. 62–65): very long and cylindrical, with one side connected to body wall by robust muscular bands. Internal surface as in vagina and penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 9). Penial retractor long, passing above right ommatophore retractor which is consequently free of penial complex and vagina.

Mating: no data available.

Remarks

Sardovitrina is a monotypic genus, recently described by Manganelli & Giusti (2005) for a species from Sardinia (western Mediterranean) having a scheme of the distal genitalia (long to very long free oviduct, vagina and genital atrium; short to very short penial complex) reminescent of some Macaronesian vitrinids (*Plutonia atlantica* and some species of *Madeirovitrina* and *Azorivitrina*) and sharing the same relationships between the right ommatophore retractor and the distal genitalia with species of *Oligolimax*, *Semilimacella* and *Vitrina.* Indeed, MP and AP showed that *Sardovitrina* constitutes a monophyletic group with these genera, supported by an exclusive synapomorphy, namely the course of the penial retractor.

Trochovitrina Boettger, 1880

Type Species: *Vitrina (Trochovitrina) subcarinata* Boettger, 1880, by monotypy. *Vitrina subcarinata* is a junior synonym of *Lampadia lederi* Boettger, 1878.

Material Examined

Trochovitrina lederi (Boettger, 1878) – Azerbaijan: "Lenkoran lowland and Talysh Mountains", A. A. Schileyko leg. 24.3.1965 (2 spirit specimens, ZMMU Lc-19985).

Diagnosis

Snail-like vitrinids characterized by penial retractor muscle arising from diaphragm and ending on penial complex apically (path unknown); stimulator consisting of glandula amatoria with complete external glandular covering (papilla of glandula amatoria apparently missing); penial complex with penial gland and without penial sheath; vas deferens entering penial complex proximally; internal structures of penial complex comprising only main pilaster, consisting of large, not distally lamellate, proximal glandular roll and robust distal muscular pleat, ending before genital atrium.

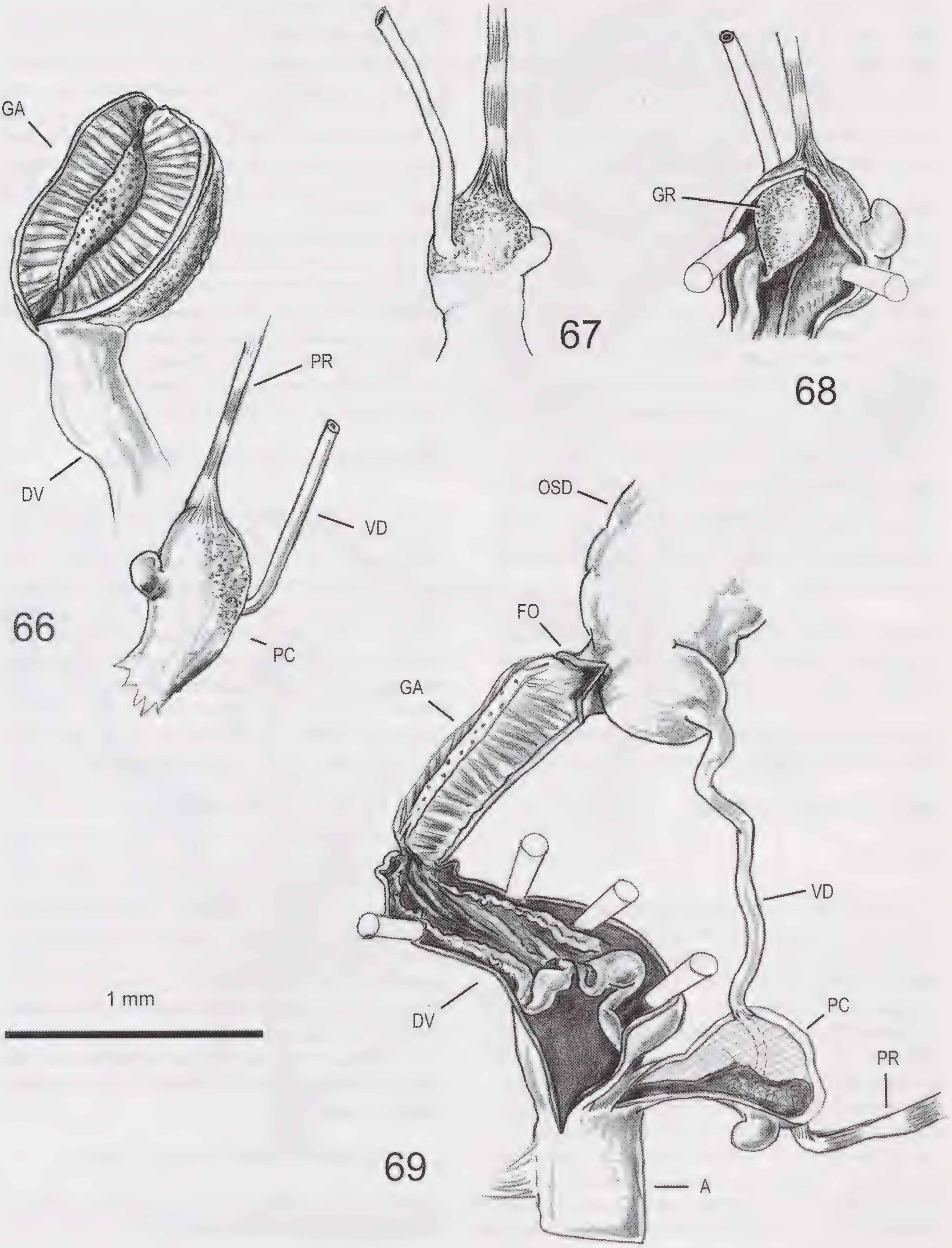
Description of Type Species

Shell: trochiform, translucent, thin and fragile, conical-depressed, sharply angled, with approximately 3.0 whorls; last whorl expanded; aperture moderately wide with margin not bordered by periostracal fringe; columellar margin normal; umbilicus open, punctiform; protoconch prominent, with fine, closely spaced, radial riblets and no evidence of pitting; teleoconch with fine, closely and regularly spaced, slightly wavy, radial riblets (Schileyko, 2003: fig. 1908A).

Body: no data available; it may possibly be withdrawn completely into shell, since shell lobes are probably very small (Hausdorf, 1995: 71).

Radula: dichoglossan; marginal teeth partly pluricuspid (Hausdorf, 1995: table 1).

Female Distal Genitalia (Figs. 66, 69): free oviduct short and wide. Bursa copulatrix and its duct apparently absent. Vagina long, with large bulbous glandula amatoria constituting half its



FIGS. 66–69. *Trochovitrina lederi* (Boettger, 1878) from "Lenkoran lowland and Talysh Mountains" (Azerbaijan), A. A. Schileyko leg. 24.3.1965. FIG. 66: Partial distal genitalia; FIG. 67: Detail of penial complex; FIG. 68: Internal structure of penial complex; FIG. 69: Internal structure of female distal genitalia and section of penial complex.

length. Glandula amatoria with thin muscular wall, almost completely covered by external glandular layer; papilla of glandula amatoria absent (?). Internal surface of distal vagina with mainly longitudinal pleats, two of which longer, more evident and wavy, ending near genital atrium as raised and rolled crests.

Male Distal Genitalia (Figs. 66–69): penial complex small and club-like, with small knob-like diverticulum at about half its length, with penial gland and without penial sheath. Penial gland covering side of proximal penial complex facing vagina. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens medium in length, entering penial complex proximally, thus dividing it into short pointed flagellum and penis proper. Internal structures of penial complex comprising main pilaster only which consists of large proximal glandular roll, not distally lamellate, and robust distal muscular pleat, ending before genital atrium. Vas deferens opening on glandular roll at about half its length. Internal surface smooth.

Genital Atrium (Fig. 69): long with one side connected to body wall by thin muscular bands. Internal surface smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: unknown.

Mating: no data available.

Remarks

Trochovitrina was established by Boettger (1880) as a subgenus of *Vitrina*. Later it was considered a junior synonym of *Gallandia* by Baker (1929), Thiele (1931) and Zilch (1959).

Schileyko (1986) resurrected it as a valid monospecific genus characterized by very short free oviduct, absence of bursa copulatrix and its duct, structure of wall of glandula amatoria, absence of papilla of glandula amatoria and penial complex with small subapical tubercle.

In a revision of Turkish vitrinids, Hausdorf (1995) did cladistic analysis to elucidate the relationships of *T. lederi* with *Gallandia annularis* and *G. paulucciae* using *Insulivitrina* as outgroup. He found that these three taxa constitute a monophyletic group (*G. annularis* (*G. olympica*, *T. lederi*)) and consequently regarded *Trochovitrina* as a junior synonym of *Gallandia* (*Gallandia* is a junior synonym of *Oligolimax*).

Our anatomical study on the specimen already studied by Schileyko (1986: 130–131, fig. 4; 2003: 1479, fig. 1908) (and consequently highly fragmented) has largely confirmed his anatomical description, particularly the absence of bursa copulatrix and papilla of glandula amatoria. A few details must be added: presence of penial gland on one side of proximal penial complex; presence of a main pilaster consisting of a proximal glandular roll and a robust distal muscular pleat.

Anatomical study of other specimens is needed to detect important features such as relationships between penial retractor and right ommatophore retractor and to confirm absence of bursa copulatrix and papilla of glandula amatoria.

Pending more detailed investigation we leave *Trochovitrina* as a distinct genus (instead of a junior synonym of *Oligolimax*).

Vitrinids with Diverticular Stimulator

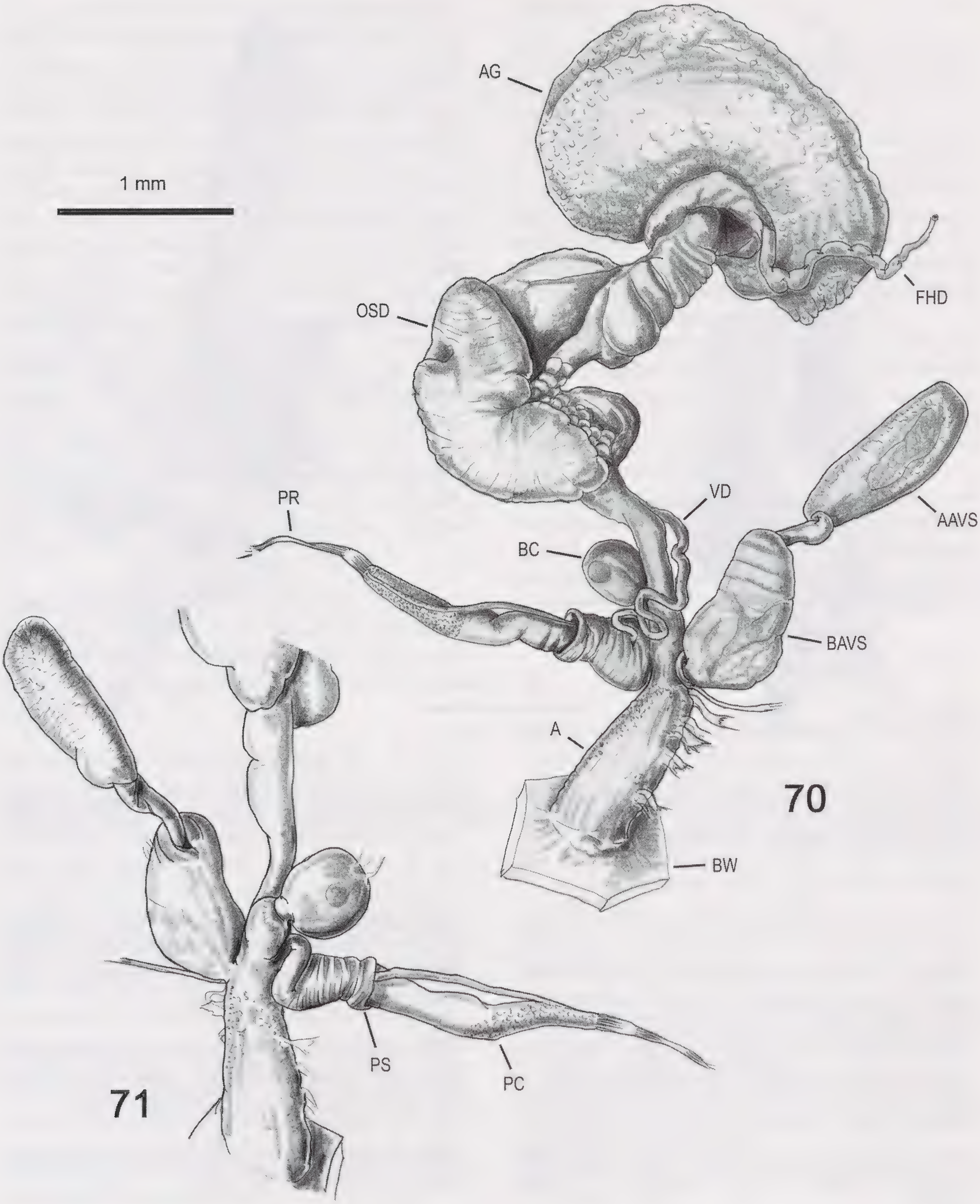
The vitrinids with diverticular stimulator include four taxa of the genus group (*Hessemilimax*, *Semilimacella*, *Semilimax* and *Vitrinobrachium*). Our phylogenetic analyses suggested that they belong to three different lineages with unresolved basal relationships: the first was *Vitrinobrachium*, the second *Hessemilimax* and *Semilimax* and the third *Semilimacella* and other taxa. The *Vitrinobrachium* clade was present in all analyses and was one of the most supported by MP. The clade including *Hessemilimax* and the three species of *Semilimax* was present in some analyses; the relationships between its members were not resolved, though MP supported a subclade consisting of *Semilimax pyrenaicus* and *S. semilimax*. The relationships of *Semilimacella* were more controversial: the two species might constitute a monophyletic (see BI) or paraphyletic group belonging to a larger monophyletic group including other taxa (species of *Oligolimax*, *Sardovitrina* and *Vitrina*) on the basis of the exclusive disposition of the penial retractor above the right ommatophore retractor (see AP).

Hessemilimax Schileyko, 1986

Type Species: *Vitrina kotulae* Westerlund, 1883, by original designation.

Material Examined

Hessemilimax kotulae (Westerlund, 1883) – Italy: Val di Tesero, Alpe di Pampeago, Rifugio Pampeago (Tesero, Trento), M. Bodon leg. 25.9.1994 (2 spirit specimens, FGC).



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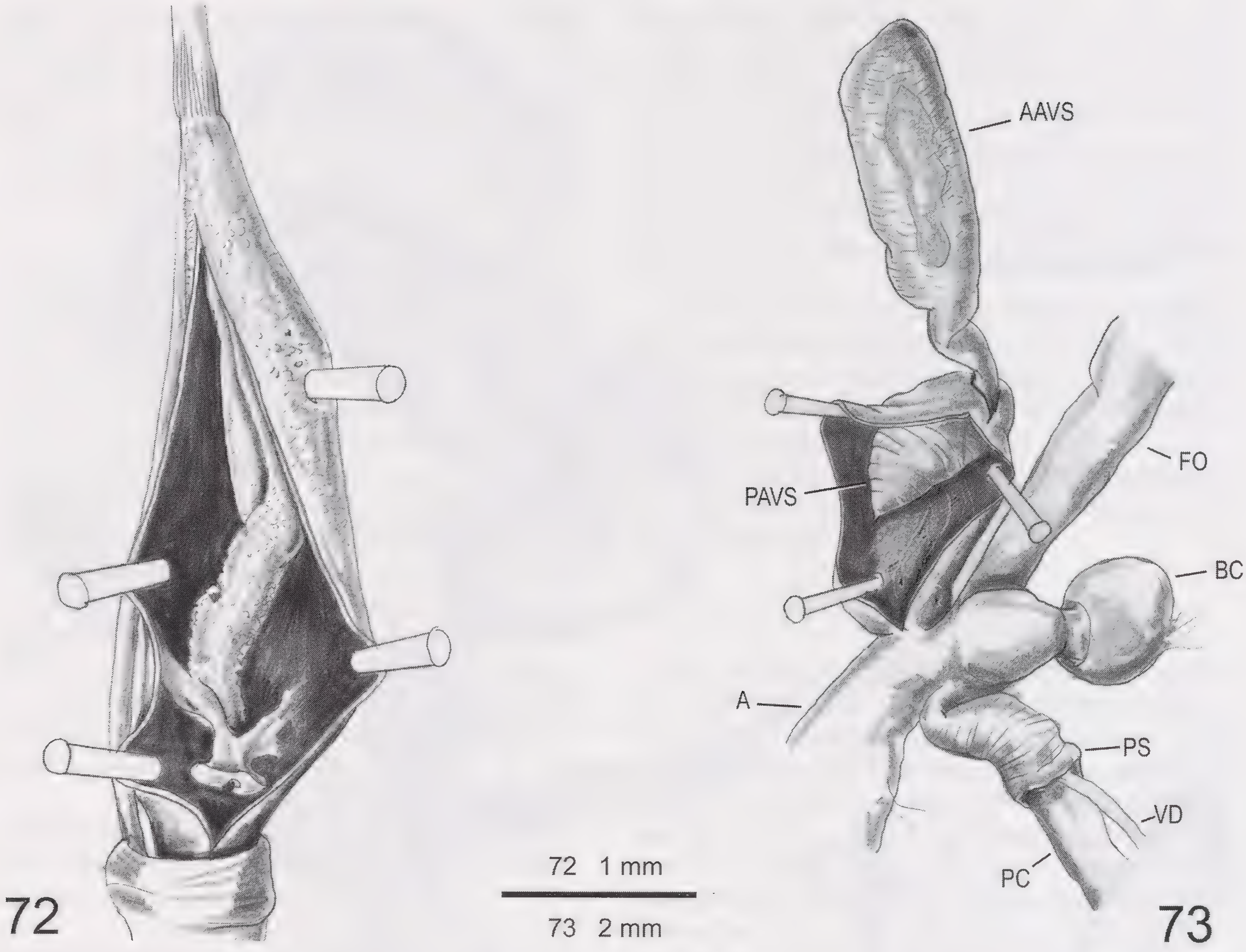
FIGS. 70, 71. Genitalia of a specimen of *Hessemilimax kotulae* (Westerlund, 1883) from Val di Tesero, Alpe di Pampeago, Rifugio Pampeago (Venetum, Italy), M. Bodon leg. 25.9.1994.

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically; stimulator atrial-vaginal, consisting of blind sac with apical portion covered by spongy glandular tissue and more or less invaginated into basal and internal



FIGS. 72, 73. *Hessemilimax kotulae* (Westerlund, 1883) from Val di Tesero, Alpe di Pampeago, Rifugio Pampeago (Venetum, Italy), M. Bodon leg. 25.9.1994. FIG. 72: Internal structure of penial complex; FIG. 73: Atrial-vaginal stimulator.

structures comprising only papilla of stimulator; penial complex with penial gland and penial sheath; vas deferens contacting penial complex distally, traversing penial sheath, running along penial complex and entering it subapically; internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters; main pilaster consisting of slender, not distally lamellate, proximal glandular roll and very short distal muscular pleat ending in sort of π - shaped papilla.

Description of Type Species

Shell: vitriniform very dilated, glossy, thin and fragile, depressed, with approximately 1.8 whorls; last whorl extremely expanded; aperture very wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin cut-away; umbilical area widely open; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 6, fig. 4a, 4b).

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed.

Radula: dichoglossan; marginal teeth unicuspid (Soós, 1917: 102, fig. 78).

Female Distal Genitalia (Figs. 70, 71, 73): free oviduct short and wide. Bursa copulatrix medium in size and sac-like, with short, slender duct, initially sligthly flared. Vagina very short, without glandula amatoria or other internal structures and without external glandular covering.

Male Distal Genitalia (Figs. 70–73): penial complex large and finger-like, with penial gland

and penial sheath. Penial gland covering most of proximal penial complex. Penial sheath long, curled up, arising from end of penial complex and enveloping almost all distal penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens long, contacting penial complex distally, then traversing penial sheath, running along surface of penial gland and entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters. Main pilaster consisting of slender proximal glandular roll, not distally lamellate, and very short distal muscular pleat ending in sort of π-shaped papilla. Smaller pilaster (branch of main pilaster or accessory pilaster?) glandular, arising side by side with larger pilaster and ending about half its length. Vas deferens opening not visible, probably level with initial part of larger pilaster. Internal surface smooth.

Genital Atrium (Figs. 70, 71, 73): long and cylindrical, with sparse glandular covering and one side connected to body wall by thin muscular bands. Internal surface with sparse spongy tissue.

Atrial-Vaginal Stimulator (Figs. 70, 71, 73): very large and long, inserted between vagina and genital atrium, opposite to side entered by penial complex, and consisting of basal and apical portions, with apical more or less invaginated into basal. Basal portion sac-like, without external glandular covering. Apical portion with slender invaginated part, without external glandular covering and free part sac-like with spongy external glandular covering. Transverse sections of apical portion reveal absence of internal glandular layer equivalent to that present in glandula amatoria. Internal structures consisting of papilla of stimulator (conical structure absent). Papilla of stimulator short to long, conical, pointed, open at tip, protruding into basal portion. Internal surface of basal portion smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults. Penial retractor long, passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which consequently appears free of penial complex and vagina.

Mating: no data available.

Remarks

The monotypic *Hessemilimax* was introduced by Schileyko (1986) and apparently not regarded as valid by Hausdorf (2002).

Species of *Hessemilimax* and *Semilimax* share an atrial-vaginal stimulator composed of basal and apical portions, the apical being more or less invaginated into the basal portion which contains a rather large, long papilla. The stimulator is similar in general organization to the vaginal glandula amatoria but differs due to absence of an internal glandular layer.

Hessemilimax includes only one species (*H. kotulae*) and is distinguished from *Semilimax* by a long finger-like penial complex, a distinctive final part of main pilaster and absence of chitinous horn-like structure inside papilla of atrial-vaginal stimulator.

Some analyses indicated that *Hessemilimax* constitutes a monophyletic group with the species of *Semilimax* (Table 3). However, the basal relationships of this clade remained unresolved, like those between *Hessemilimax* and species of *Semilimax*.

Semilimacella Soós, 1917

Type Species: *Vitrina (Semilimacella) velebitica* Soós, 1917, by monotypy. *Vitrina velebitica* Soós is a junior synonym of *Vitrina reitteri* Boettger, 1880, according to Forcart (1960) and Schileyko (2003). In turn, *Vitrina reitteri* is a subspecies of *Semilimacella bonelli* (Targioni Tozzetti, 1873) according to Forcart (1960) and Schileyko (2003).

Synonyms

Balcanovitrina Osanova & Pintér, 1968. Type Species: *Balcanovitrina dojtshini* Osanova & Pintér, 1968, by monotypy.

Targionia Hesse, 1923 (*non* Signoret, 1869; Hemiptera). Type Species: *Vitrina bonelli* Targioni Tozzetti, 1873.

Tozzettia Hesse, 1924. New name for *Targionia* Hesse, 1923.

Material Examined

Semilimacella bonelli (Targioni Tozzetti, 1873) – Italy: Monti Reatini, Vallonina (Rieti), 33TUH30, F. Giusti leg. 20.11.1969 (2 spirit specimens, FGC); Monti Sibillini, Val Canatra (Norcia, Perugia), 33TUH54, F. Giusti & G. Manganelli leg. 13.9.1988 (1 spirit specimen, FGC).

Semilimacella cephalonica (Rähle, 1980) – Greece: Cefalonia Island, Ainos, W. Rähle leg. 24.9.1978 (4 spirit specimens, SMF 252354).

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, passing above right ommatophore retractor and ending on penial complex apically; stimulator atrial or vaginal, consisting of blind sac with apical portion covered by spongy glandular tissue and internal structures comprising one or two conical chitinous (at least in *S. bonelli*) structures; penial complex with penial gland and penial sheath; vas deferens contacting penial complex distally, traversing penial sheath, running along penial complex and entering it apically or proximally; internal structures of penial complex comprising only main pilaster consisting of large glandular roll divided into small, not distally lamellate, proximal portion and large medial-distal portion (at least in *S. bonelli*).

Description of Type Species

Shell: vitriniform slightly dilated, glossy, thin and fragile, depressed, with approximately 3.0 whorls; last whorl well expanded; aperture wide with basal margin bordered by narrow periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Giusti et al., 1985: pl. 10, fig. H).

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed.

Radula: dichoglossan; marginal teeth pluricuspid (Targioni Tozzetti, 1873: 20, pl. 6, fig. 3; Giusti, 1973: 465, pl. 4, figs. 1–3).

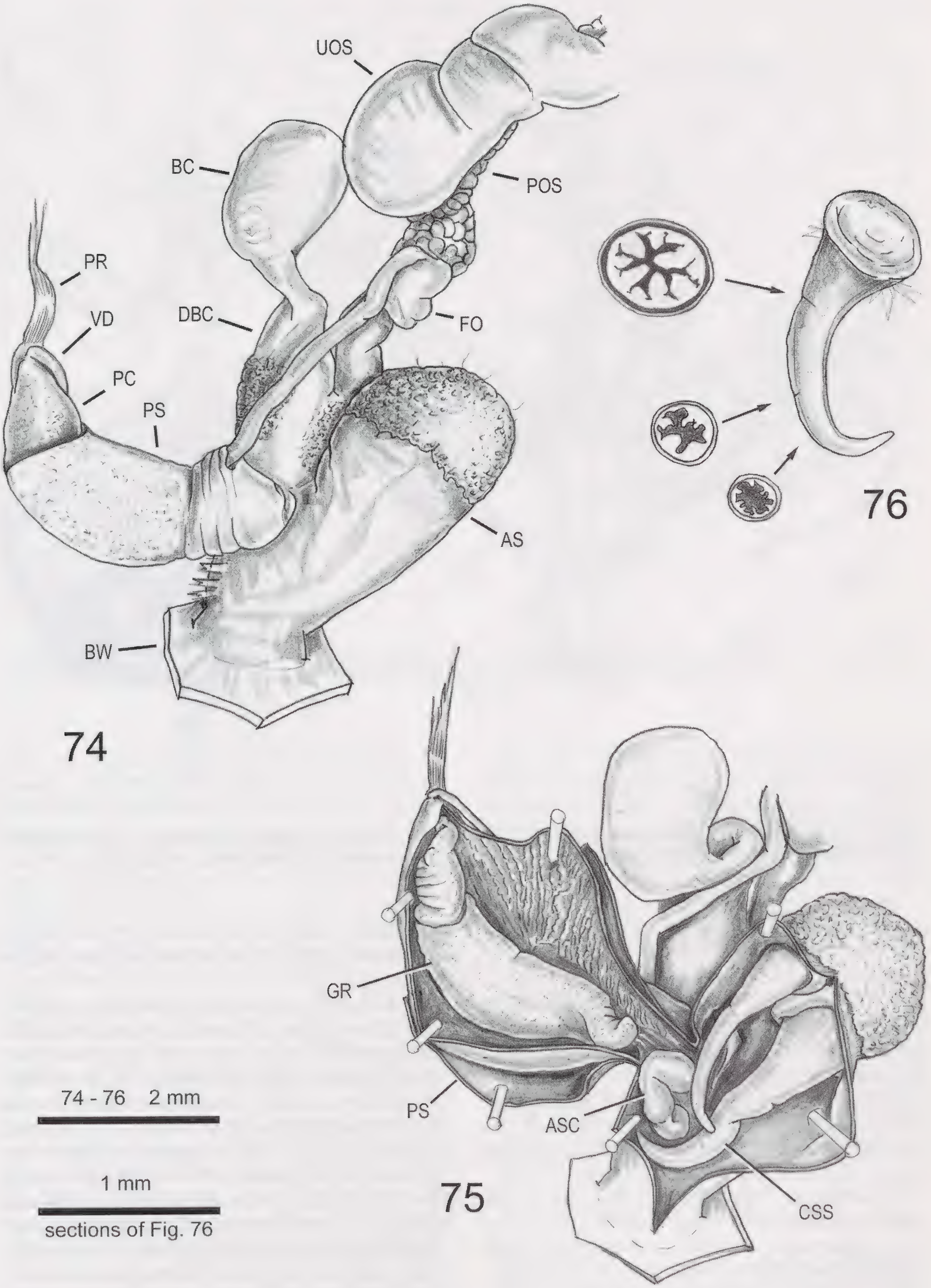
Female Distal Genitalia (Figs. 74, 75): free oviduct short and wide. Bursa copulatrix medium in size and sac-like, with short to medium, more or less slender duct, initially very flared. Vagina short, without glandula amatoria or other internal structures, but with external glandular covering on one side and initial part of duct of bursa copulatrix.

Male Distal Genitalia (Figs. 74, 75): penial complex large and club-like, with penial gland and penial sheath. Penial gland covering most of penial complex. Penial sheath long, arising from end of penial complex and enveloping almost all penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens long, contacting penial complex distally, then traversing penial sheath, running along surface of penial gland and entering penial complex apically near insertion of penial retractor. Internal structures of penial complex comprising main pilaster only which consists of large glandular roll divided into small whitish proximal portion, slightly lobate, not distally lamellate, and large but progressively more slender medial–distal portion, ending before genital atrium. Vas deferens opening in small slit, level with proximal penial complex. Internal surface with thin parallel segmented mainly transverse pleats.

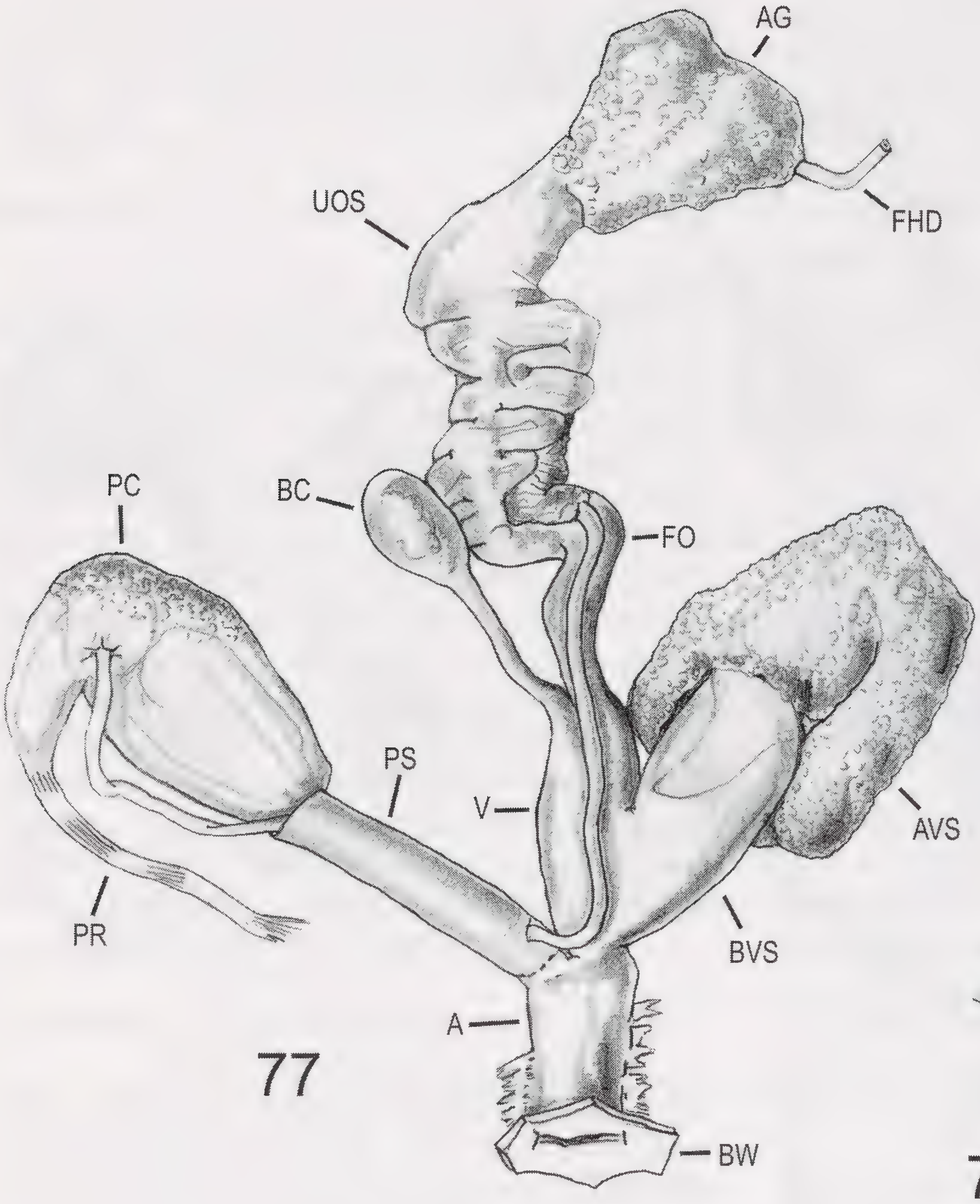
Genital Atrium (Figs. 74, 75): short, with large club-like atrial stimulator and one side connected to body wall by thin muscular bands. Internal structures consisting of proximal semicircular crest forming sort of bulkhead level with tips of horn-like structures of atrial stimulator and close to opening of duct of bursa copulatrix. Internal surface smooth.

Atrial Simulator (Figs. 74–76): large and club-like, entering genital atrium opposite to side entered by penial complex, and consisting of basal and apical portions. Basal portion tubular, without external glandular covering. Apical portion cap-like, with thick spongy external glandular covering and internal compact mass of tissue. Internal structures comprising two conical structures ("brachia copulatoria"; Schileyko, 2003), similar in shape but different in size, with rather resistant yellowish subtransparent chitinous walls and internal cavity partly filled with whitish material; conical structures with bases lodged into compact mass of tissue filling apical portion (but not firmly connected to it being rather easily removed) and pointed tips reaching genital atrium. Internal surface of basal portion smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 11). Penial retractor long, passing above right ommatophore retractor which is consequently free of penial complex and vagina (right ommatophore retractor is below penial complex and above atrial stimulator).



FIGS. 74–76. *Semilimacella bonelli* (Targioni Tozzetti, 1873) from Monti Reatini, Vallonina (Latium, Italy), F. Giusti leg. 20.11.1969. FIG. 74: Distal genitalia; FIG. 75: Internal structure of penial compex and vagina; FIG. 76: An isolated conical structure.



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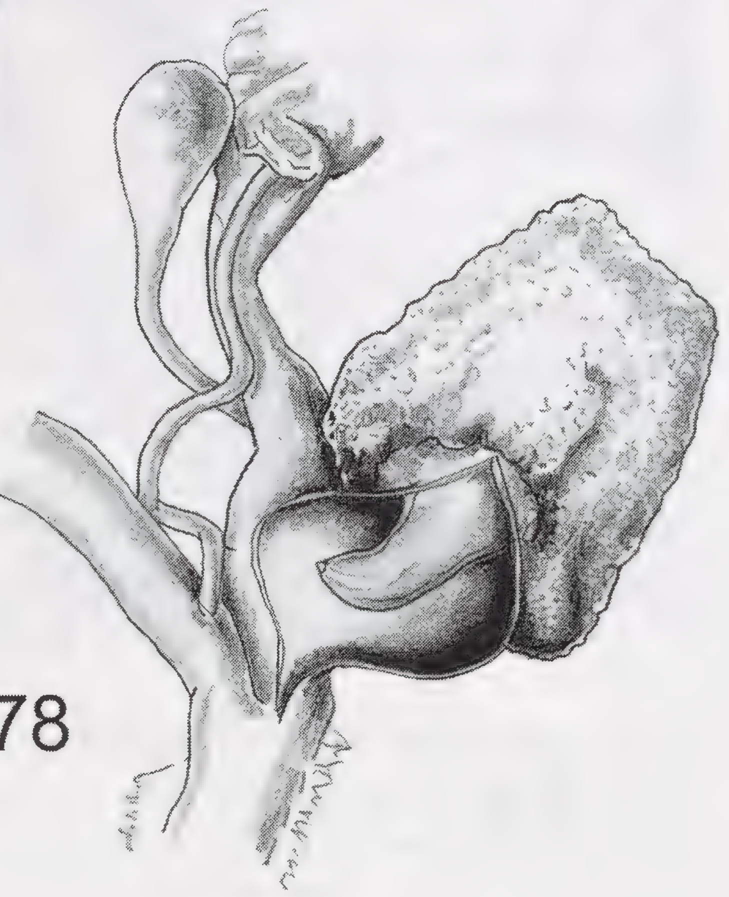

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FIGS. 77, 78. FIG. 77: Distal genitalia; FIG. 78: Internal stucture of vaginal stimulator. *Semilimacella cephalonica* (Rähle, 1980) from Cefalonia Island, Ainos (Greece), W. Rähle leg. 24.9.1978.

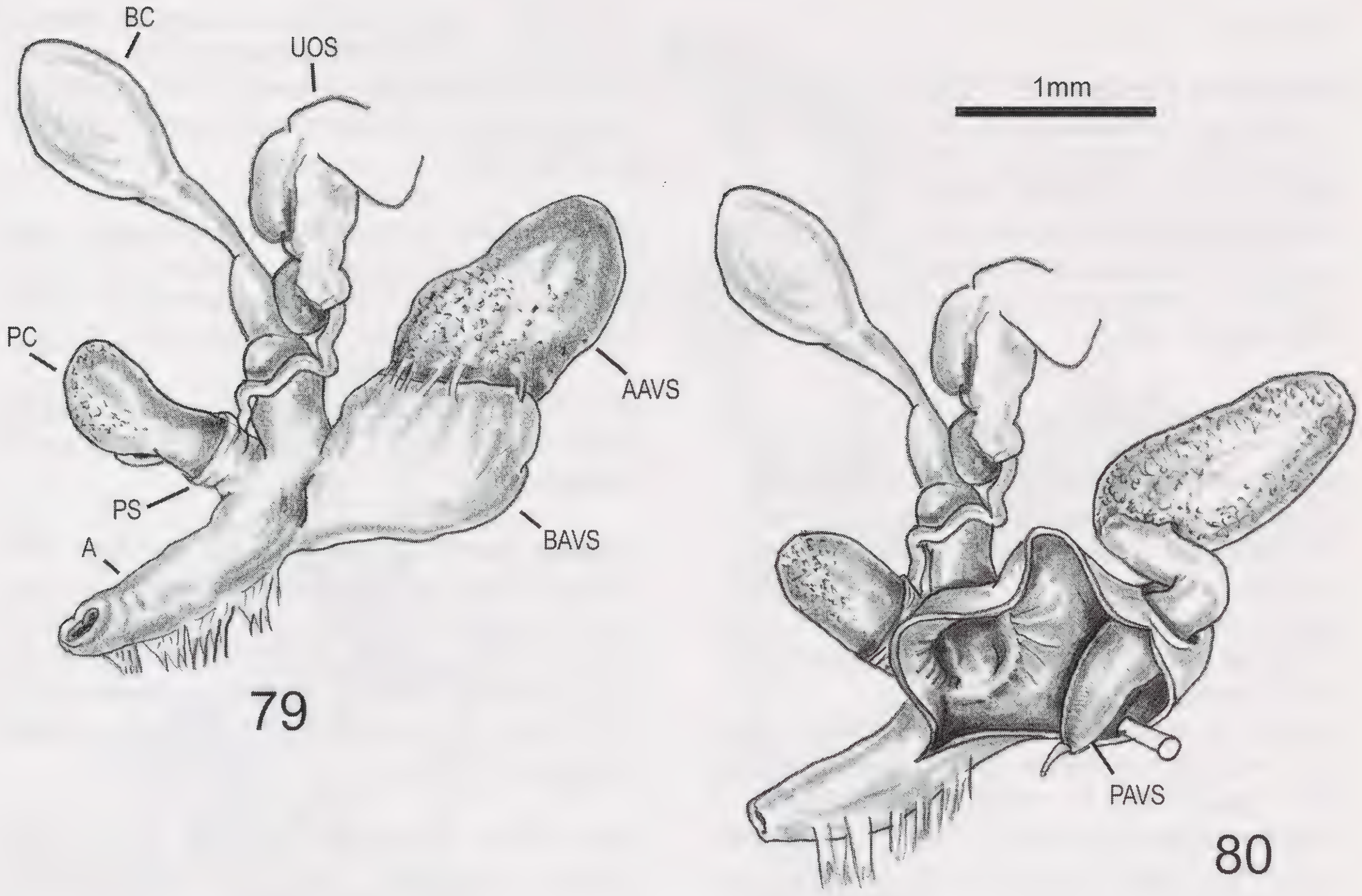
Mating: no data available.

Remarks

Semilimacella was introduced by Soós (1917) as a subgenus of *Vitrina*. It has since been regarded as a junior synonym of *Semilimax* (Baker, 1929; Thiele, 1931), a subgenus of *Vitrina* (Zilch, 1959), a junior synonym of *Oligolimax* (Forcart, 1965) and finally a distinct genus (Damjanov & Likharev, 1975).

According to Hausdorf (2002), *Semilimacella* includes only three species from the Alps, Apennines, Balkans and central Europe (major anatomical references on which are: Targioni Tozzetti, 1873; Forcart, 1960; Rähle, 1980; Mildner, 1982) and is the sister group of *Vitrina*; in turn *Semilimacella* + *Vitrina* are the sister group of (*Eucobresia* (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*)))).

The atrial stimulator of *Semilimacella* is completely different from that of *Semilimax* and *Hessemilimax*, the only other vitrinids with an atrial or atrial-vaginal stimulator: it is shorter and its apical part is not invaginated into the basal portion. Its apical part is somewhat bilobate with a thick external layer of glandular tissue and is not hollow internally. Tissue filling the apical part is compact, muscular in appearance and lodges the base of two conical structures which have rather long, tapered pointed tips extending into the lumen and as far as the genital atrium. These two structures have cuticularized, thin but resistant chitinous-like walls and contain whitish condensed material adhering to internal



FIGS. 79, 80. *Semilimax semilimax* (Férussac, 1802) from Plitvička Jesera (Croatia), A. Minelli leg. 13.4.1968. FIG. 79: Distal genitalia; FIG. 80: Internal structure of distal genitalia.

wall surfaces; these horn-like structures are easily detached from their muscular support and their base is closed, so that there is no evidence of direct communication between their lumen and the glandular mass inside "cul de sac" of stimulator. Nature and origin of whitish material is unknown.

Two other species, *S. carniolica* (Boettger, 1884) and *S. cephalonica*, are assigned to *Semilimacella* by Hausdorf (2002), but many details of their anatomy are unclear. Remarkably, their stimulator is sometimes reported as distally bilobate and vaginal (Rähle, 1980; Mildner, 1982; Schileyko, 2003). We tried to re-examine them but unfortunately our attempt to find spirit specimens of *S. carniolica* were unsuccessful. Re-examination of *S. cephalonica* (Figs. 77, 78) confirmed most details reported by Rähle (1980) and showed that this species and *S. bonelli* share some characters (penial sheath; penial retractor passing above right ommatophore retractor; club-like stimulator with apical glandular cap), but differ in many others (duct of bursa copulatrix initially very flared in *S. bonelli*, not flared in *S. cephalonica*; vas deferens entering penial complex apically in *S. bonelli*, proximally in *S. cephalonica*; stimulator atrial with a small, cap-like, spongy external glandular covering and two internal conical chitinous structures in *S. bonelli*, vaginal with large cap-like spongy external glandular covering and only one internal conical structure in *S. cephalonica*).

Only BI recovered the two *Semilimacella* species as a monophyletic group whereas AP suggested that they could be a paraphyletic group (*S. cephalonica* (*S. bonelli*, *V. pellucida*)) belonging to a larger monophyletic group, including three other species (*Oligolimax annularis*, *O. musignani* and *Sardovitrina polloneriana*) on the basis of the exclusive disposition of the penial retractor above the right ommatophore retractor.

Semilimax Stabile, 1859

Type Species: *Vitrina elongata* Draparnaud, 1805, by subsequent designation (Fischer in: Paulucci, 1878) (*V. elongata* is a junior synonym of *Helix semilimax* Férussac, 1802).

Synonyms

Chlamydea Westerlund, 1886. Type Species: *Vitrina bicolor* Westerlund, 1886, by monotypy (according to Bank et al., 2001).

Vitrinopugio von Ihering, 1892. Type Species: *Vitrina elongata* Draparnaud, 1805, by subsequent designation (Hesse, 1923) (*V. elongata* is a junior synonym of *Helix semilimax* Férussac, 1802).

Material Examined

Semilimax carinthiacus (Westerlund, 1886) – Italy: Laghi di Fusine (Tarvisio, Udine), A. Minelli leg. 22.8.1972 (1 spirit specimen, FGC).

Semilimax pyrenaicus (Férussac, 1821) – France: Lac d'Oredon, G. Marcuzzi leg. (2 spirit specimens, FGC).

Semilimax semilimax (Férussac, 1802) – Austria: Hochkönig, Dienten, Sessellift-Bergstation (Salzburg), O. Paget leg. 30.7.1973 (2 spirit specimens, NHMW 74239); Saalbach, bei Haus Hapimag (Salzburg), O. Paget leg. 4.7.1972 (2 spirit specimens, NHMW 77954). Croatia: Plitvička Jesera, A. Minelli leg. 13.4.1968 (1 spirit specimen, FGC).

Diagnosis

Semislug-like vitrinids characterized by absence of penial retractor (but in one species long, arising from diaphragm, running in front of right optic nerve, below right ommatophore retractor and ending on penial complex subapically); stimulator atrial-vaginal, consisting of blind sac with apical portion covered by spongy glandular tissue and more or less invaginated into basal and internal structures comprising only papilla of stimulator traversed by chitinous hook-like structure (absent in one species); penial complex with penial gland and penial sheath; vas deferens contacting penial complex distally, traversing penial sheath, running along penial complex and entering it subapically; internal structures of penial complex comprising only main pilaster, consisting of large, not distally lamellate, proximal glandular roll.

Description of Type Species

Shell: vitriniform very dilated, glossy, thin and fragile, depressed, with approximately 1.8 whorls; last whorl extremely expanded; aperture very wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin slightly cut-away; umbilical area open; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 6, fig. 3a–c).

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed (Falkner, 1990: fig. 2 at p. 173; 1992: fig. 2 at p. 270).

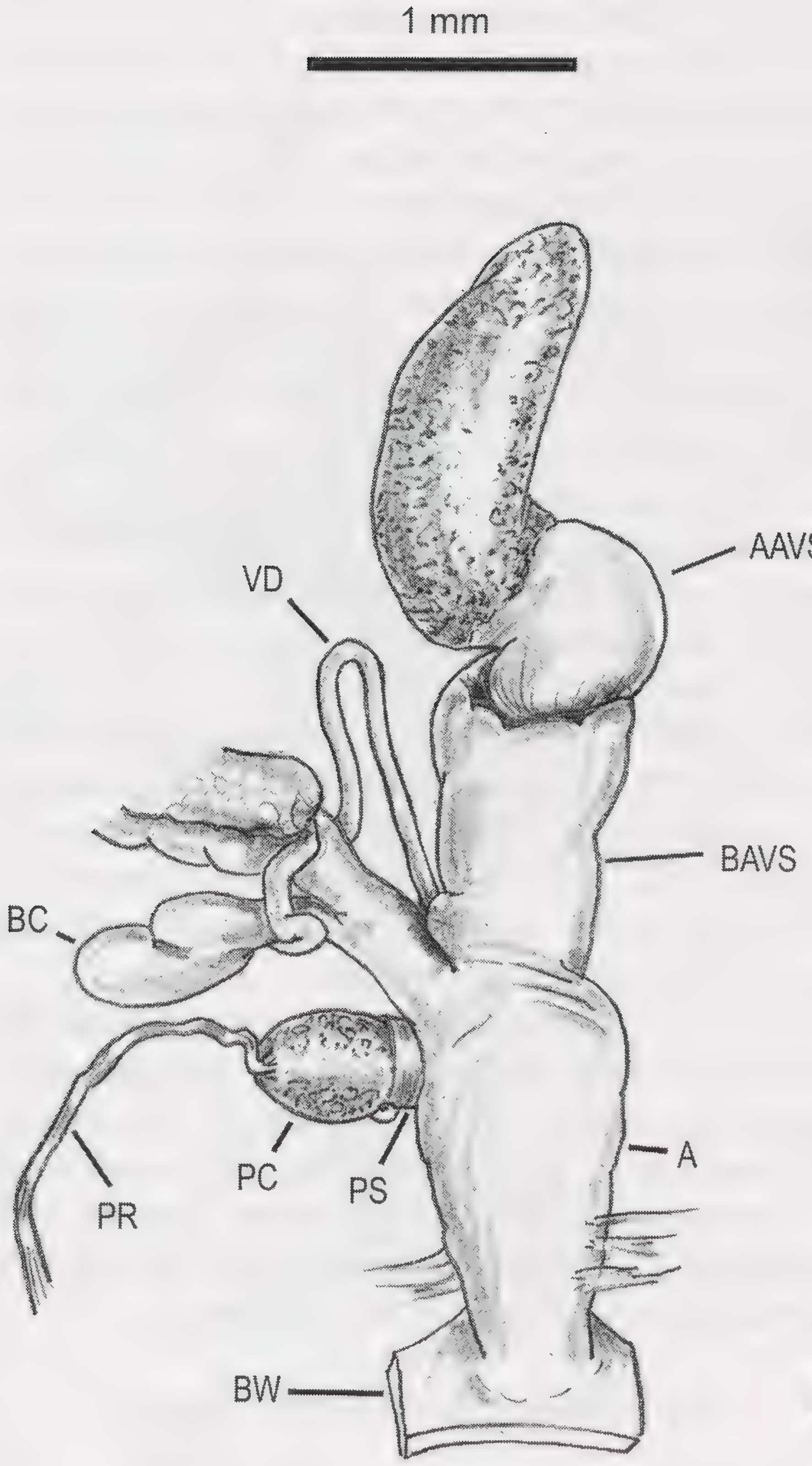
Radula: dichoglossan; marginal teeth unicuspid (Wagner, 1915: 474, pl. 11, fig. 83 a, b, as *S. elongatus*).

Female Distal Genitalia (Figs. 79, 80): free oviduct short and wide. Bursa copulatrix medium in size and sac-like, with short to medium, more or less slender duct, initially not flared. Vagina short, without glandula amatoria or other internal structures and without external glandular covering.

Male Distal Genitalia (Figs. 79, 80): penial complex very small and club-like, with penial gland and penial sheath. Penial gland covering most of proximal penial complex. Penial sheath short, arising from end of penial complex. Penial retractor absent (other *Semilimax* species have penial retractor arising from diaphragm, long, inserted apically). Vas deferens long, contacting penial complex distally, then traversing penial sheath, running along surface of penial gland and entering penial complex subapically. Internal structures of penial complex comprising main pilaster only which consists of large glandular roll, not distally lamellate, ending before genital atrium. Vas deferens opening not visible, probably level with initial part of glandular roll. Internal surface smooth.

Genital Atrium (Figs. 79, 80): long and cylindrical, with one side connected to body wall by thin muscular bands. Internal surface smooth.

Atrial-Vaginal Stimulator (Figs. 79, 80): very large and long, inserted between vagina and genital atrium, opposite to side entered by penial complex, and consisting of basal and apical portions, with apical more or less invaginated into basal portion. Basal portion sac-like or cylindrical, without external glandular covering. Limit between apical and basal portions marked by transverse groove, originating by invagination of apical into basal portion. Apical portion with invaginated part slender, without external glandular covering, and free part



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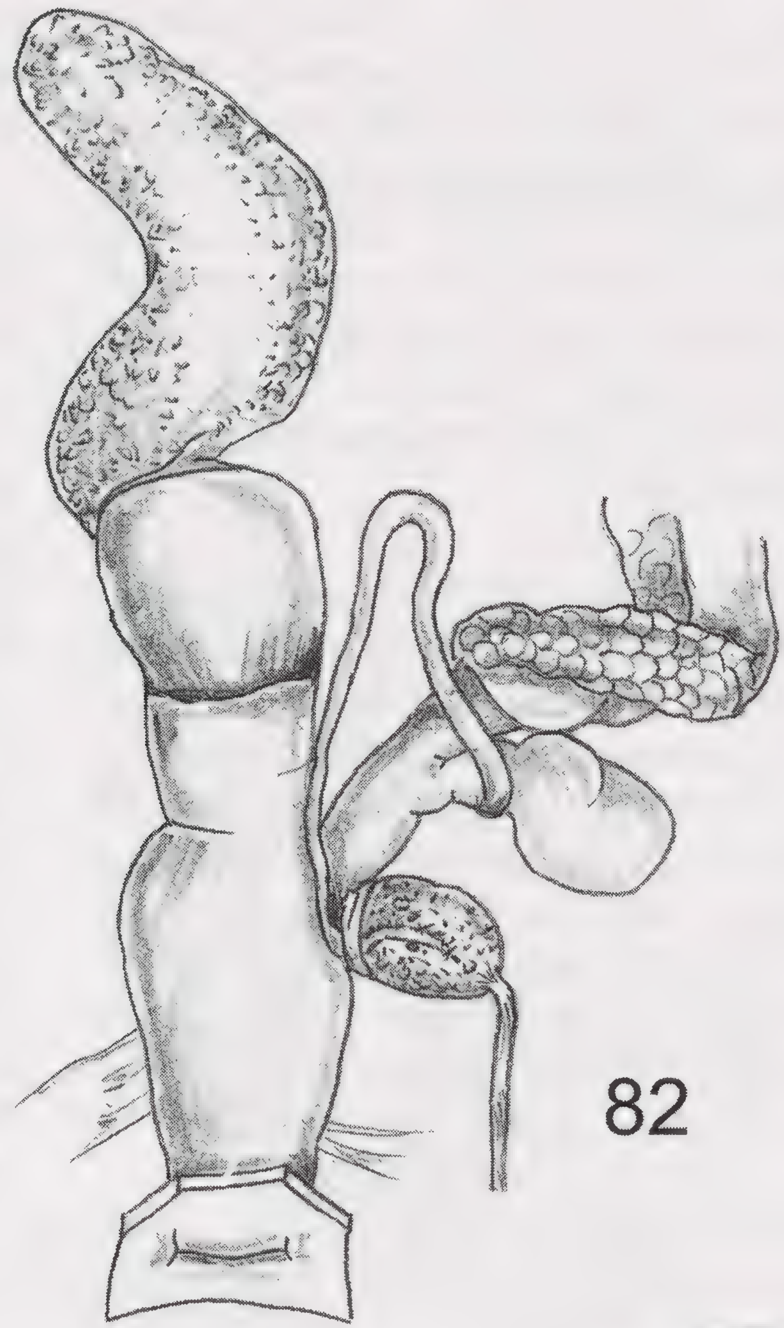

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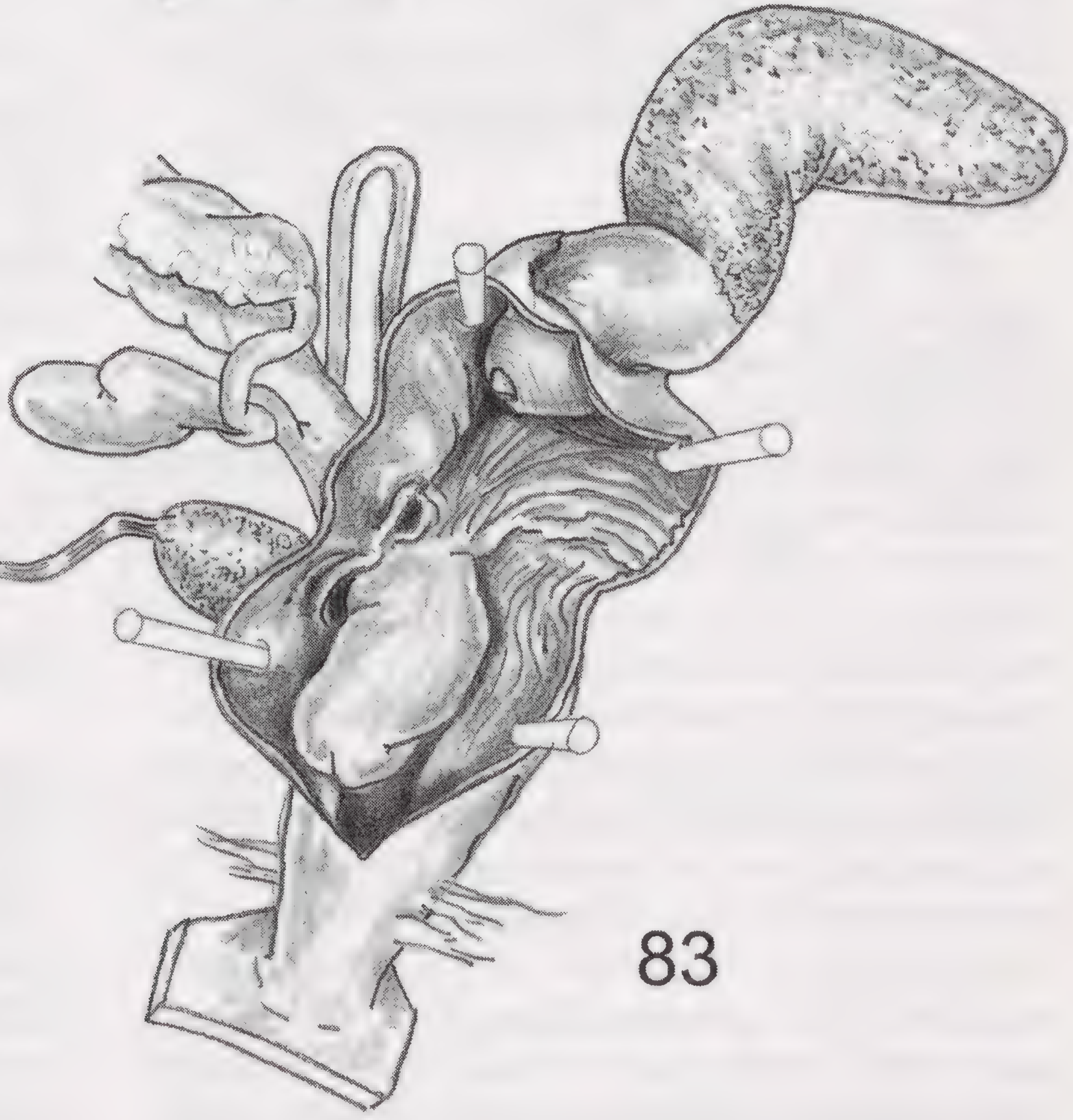

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FIGS. 81–83. *Semilimax carinthiacus* (Westerlund, 1886) from Laghi di Fusine (Friuli-Venetia Giulia, Italy), A. Minelli leg. 22.8.1972. FIGS. 81, 82: Distal genitalia; FIG. 83: Internal structure of distal genitalia.

sac-like, with thick spongy external glandular covering. Transverse sections of apical portion reveal absence of internal glandular layer equivalent to that in glandula amatoria. Internal structures consisting only of papilla of stimulator traversed by hook-like structure. Papilla of stimulator pointed, open at tip, protruding into basal portion. Hook-like structure ("Conchinspitze", Künkel, 1933; "thorn of sarcobelum", Schileyko, 2003), somewhat recalling those of *Semilimacella*, with very thin apparently chitinous walls, present inside invaginated part of apical portion; it has wide base and very slender pointed tip which usually protrudes out of stimulator papilla (Fig. 2; see also Wagner, 1915: fig. 141; Künkel, 1933: figs. 7C, 9A, 12; Schileyko, 2003: fig. 1914). Internal surface of basal portion smooth.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: due to the fact that penial retractor is absent, right ommatophore retractor is free of distal genitalia. In other *Semilimax* species (e.g., *S. carinthiacus*, Fig. 4) they are similar in juveniles and adults: penial retractor long and slender, passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which consequently appears free of penial complex and vagina.

Mating: described by Künkel (1933). After protruding out of papilla of stimulator, hook-like structure produces sucker-like structure that attaches to partner's body; everted penis inserts into vagina, transferring spermatic mass.

Remarks

Semilimax was established by Stabile (1859) with seven species originally included. Overlooking Fischer's designation (in: Paulucci, 1878) of *Vitrina elongata* Draparnaud, 1805, as the type species, Hesse (1923) considered *Semilimax* a subgenus of *Phenacolimax*, corresponding to the group of *Vitrina diaphana* (i.e., to the species now included in *Eubobresia*). Few years later, Baker (1929) corrected Hesse's misinterpretation, restoring the correct sense of this taxon. *Semilimax* was thus considered a distinct genus by all subsequent authors except Thiele (1931), who regarded it as a subgenus of *Vitrina*.

According to Hausdorf (2002), *Semilimax* includes five species from Europe (major anatomical references on which are: Boycott, 1914; Hesse, 1923; Soós, 1924; Künkel, 1933; Forcart, 1956; Schileyko, 1986; Hausdorf, 2002) and has unresolved relationships with *Vitrinobrachium* and ((*Semilimacella*, *Vitrina*) (*Eucobresia* (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*))))).

Species of *Hessemilimax* and *Semilimax* share an atrial-vaginal stimulator composed of apical sac-like glandular part and basal part, containing a rather large, long papilla. The stimulator is similar in general organization to the vaginal glandula amatoria but different due to the absence of an internal glandular layer.

Two species of *Semilimax* (*S. semilimax* and *S. carinthiacus*) have a hook-like structure (reminiscent of the conical structures of the stimulator of *Semilimacella*?) inside the invaginated portion of the apical part of the atrial-vaginal stimulator. The hook-like structure has very thin, apparently cuticularized walls, a rather large basal portion and a very slender pointed tip, lodged inside the lumen of the papilla. The tip usually protrudes from the apical opening. Davison & Mordan (2005) claimed that it is homologous to a dart.

S. pyrenaicus and *S. semilimax* lack the penial retractor (they differ for the shell shape: ear-like, with columellar margin of peristome slightly cut-away in the first; typical vitriniform, with columellar margin of peristome normal in the second). *Semilimax carinthiacus* has penial retractor and a large outgrowth inside the genital atrium, situated between openings of penis and vagina (Figs. 81–83), and is characterized by shell with rather normal columellar margin of peristome (i.e., not cut-away).

Some analyses found that species of *Semilimax* constitute a differently supported, monophyletic group with *Hessemilimax* (Table 3). The basal relationships of this clade remained unresolved like those between its members, though MP recovered a subclade consisting of *Semilimax pyrenaicus* and *S. semilimax* (Table 3).

Vitrinobrachium Künkel, 1929

Type Species: *Helicolimax breve* Férussac, 1821, by monotypy.

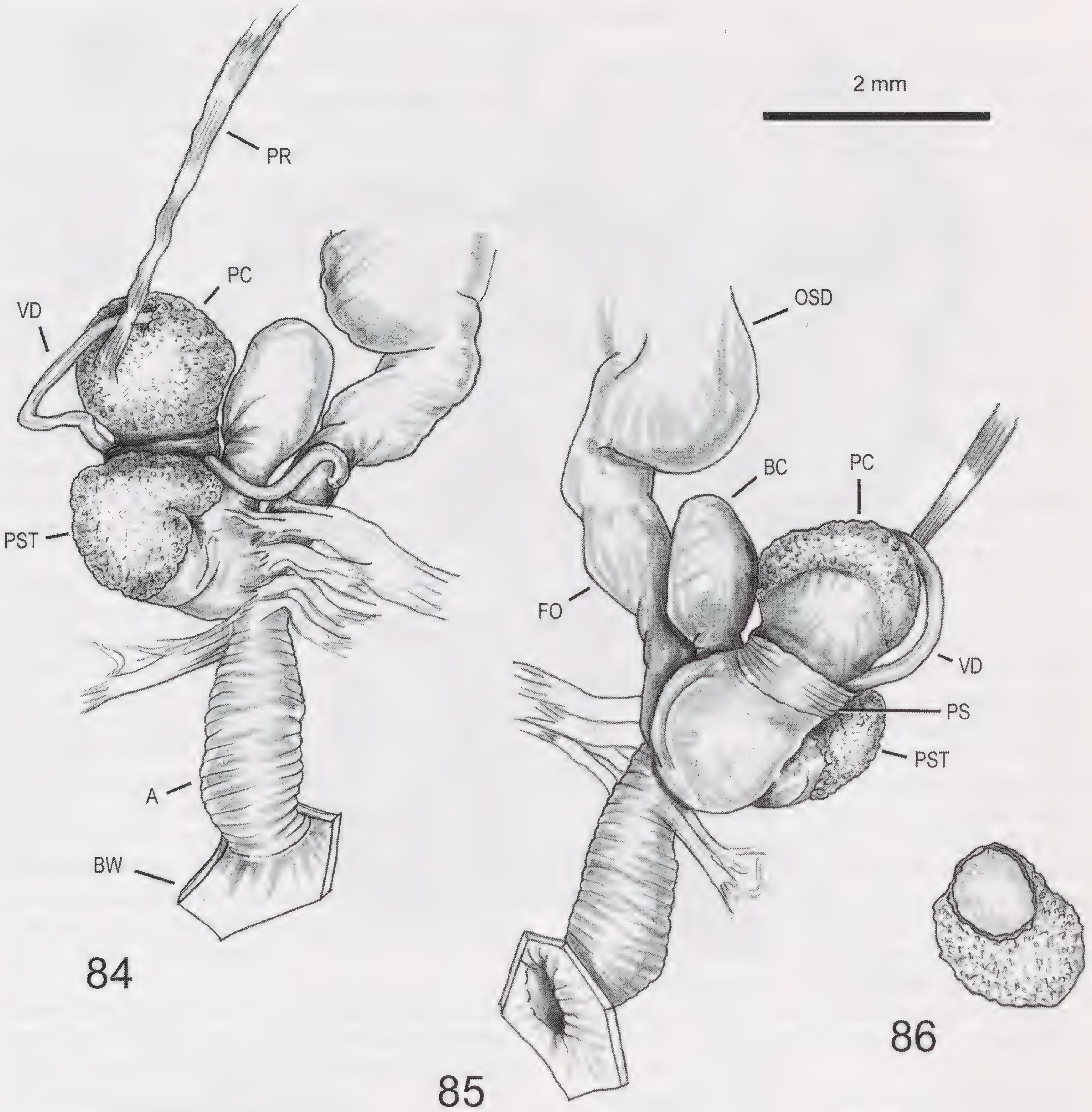
Material Examined

Vitrinobrachium baccetti Giusti & Mazzini, 1971 – Italy: Alpi Apuane, Monte Freddone (Stazzema, Lucca), 32TPP07, F. Giusti leg. 29.9.1969 (7 spirit specimens, FGC).

Vitrinobrachium breve (Férussac, 1821) – Italy: Altopiano del Cansiglio, La Crosetta (Treviso), 33TTM9901, F. Giusti leg. 11.11.1976 (2 spirit specimens, FGC). Altopiano del Cansiglio, Monte Pizzoc (Treviso), 33TTM9901, F. Giusti leg. 11.11.1976 (2 spirit specimens, FGC).

Diagnosis

Semislug-like vitrinids characterized by long penial retractor muscle arising from columellar muscle, running in front of right optic nerve, below right ommatophore retractor and ending



FIGS. 84–86. *Vitrinobrachium breve* (Férussac, 1821) from Altopiano del Cansiglio, La Crosetta (Venetum, Italy), F. Giusti leg. 11.11.1976. FIGS. 84, 85: Distal genitalia; FIG. 86: Transverse section of stimulator.

on penial complex subapically; stimulator penial, consisting of blind sac with apical portion covered by spongy glandular tissue and internal structures comprising only pedunculate sucker; penial complex with penial gland and penial sheath; vas deferens contacting penial complex medially, traversing penial sheath, running along penial complex and entering it subapically; internal structures of penial complex very distinctive, comprising sort of flat glans, divided into two semicircular tiles, and wrinkled crest.

Description of Type Species

Shell: vitriniform very dilated, glossy, thin and fragile, depressed, with approximately 1.8 whorls; last whorl extremely expanded; aperture very wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 6, fig. 2a–c).

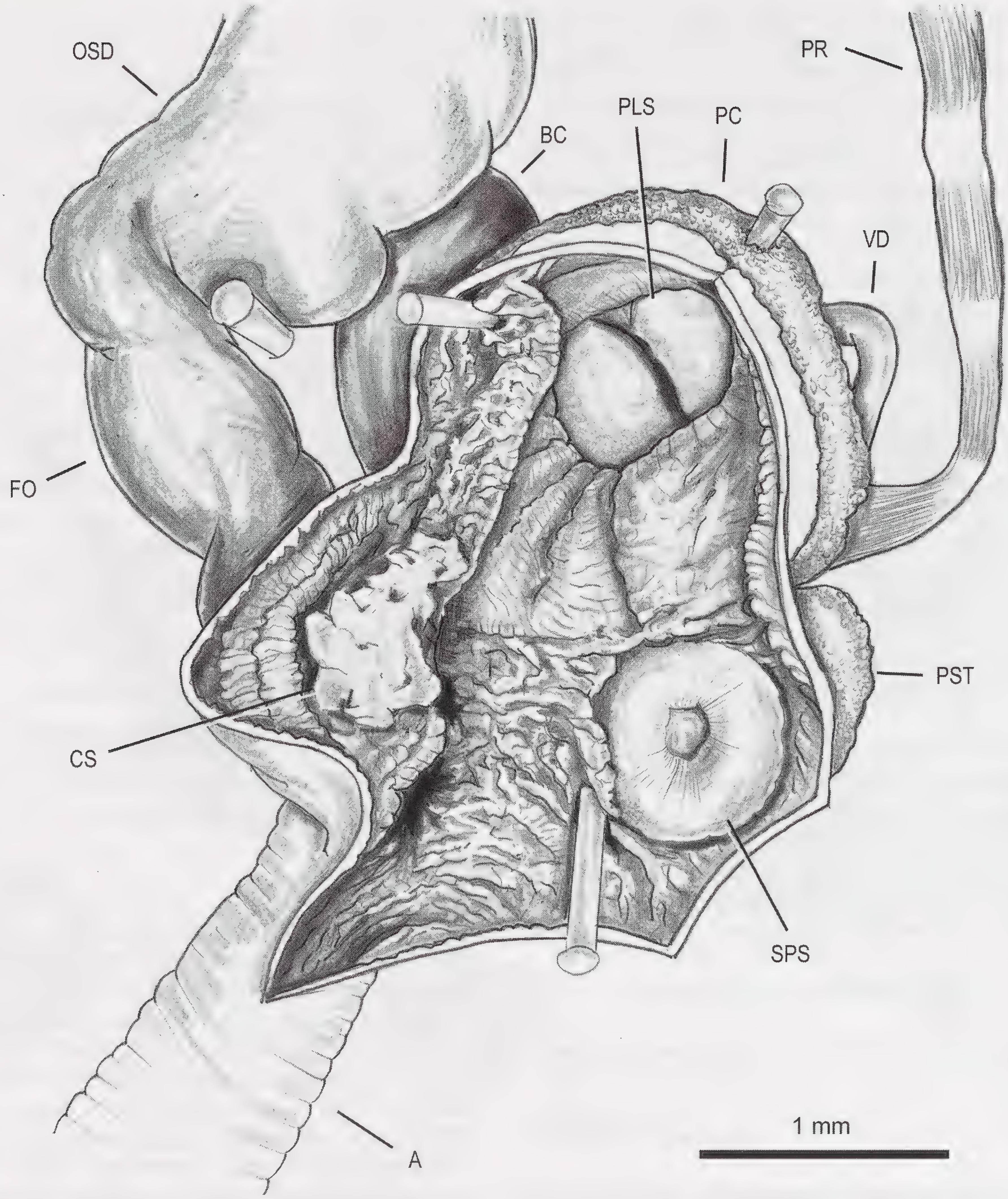


FIG. 87. Internal structure of distal genitalia of a specimen of *Vitrinobrachium breve* (Férussac, 1821) from Altopiano del Cansiglio, La Crosetta (Venetum, Italy), F. Giusti leg. 11.11.1976.

Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed (Kerney & Cameron, 1979: pl. 6, fig. 2d; Falkner, 1990: fig. 3 at p. 173; 1992: figs. 3–5 at p. 270).

Radula: dichoglossan; marginal teeth unicuspid (Wagner, 1915: 475, pl. 11, fig. 84).

Female Distal Genitalia (Figs. 84, 85, 87): free oviduct short and wide. Bursa copulatrix medium in size and oval, with short, slender to wide duct, initially not flared; bursa copulatrix duct entering distal penial complex (not proximal vagina) on side opposite to that with penial stimulator. Vagina absent.

Male Distal Genitalia (Figs. 84–87): penial complex large and globular, with penial gland, penial sheath and distally, on opposite sides, penial stimulator and bursa copulatrix. Penial gland covering most of penial complex. Penial sheath short, arising from mid penial complex. Penial retractor arising from columellar muscle, long, inserted subapically. Vas deferens long, contacting penial complex medially, then traversing penial sheath and entering penis subapically near insertion of penial retractor. Internal structures of penial complex very distinctive and therefore difficult to homologize, comprising sort of flat glans and wrinkled crest. Flat glans, divided into two semicircular tiles by rather wide groove, is located in correspondence with thick cap formed by penial gland. Wrinkled undulating crest arises close to glans and ends in proximal genital atrium. Vas deferens opening in wide groove dividing glans into two semicircular tiles. Internal surface with longitudinal, very segmented pleats, diffuse spongy tissue with crypts, and sometimes variably large masses of coagulated secretion.

Penial Stimulator (Figs. 84–87): rather large, club-like or globular, entering distal penial complex opposite to side entered by duct of bursa copulatrix and consisting of basal and apical portions. Basal portion tubular, without external glandular covering. Apical portion cap-like, with thick external glandular covering and internal compact mass of tissue. Internal structures consisting of pedunculate sucker-like structure hollow at centre, lodged into compact mass of tissue filling apical portion. Internal surface of basal portion with spongy tissue and, sometimes, variably large masses of coagulated secretion.

Genital Atrium (Figs. 84, 85, 87): long to very long and cylindrical, with one side connected to body wall by robust muscular bands. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 8). Penial retractor long, passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina.

Mating: described by Künkel (1929, 1933). Pedunculate sucker of stimulator attaches to partner's body and everted penial complex rolls up around everted stimulator; spermatic mass is attached to everted stimulator; then everted penial complex detaches from sucker peduncle and everted stimulator with spermatic mass is withdrawn inside body.

Remarks

Vitrinobrachium was introduced by Künkel (1929) as a distinct genus and has been regarded as such by all subsequent authors (Forcart, 1944; Zilch, 1959; Schileyko, 1986, 2003; Hausdorf, 2002).

According to Hausdorf (2002), *Vitrinobrachium* includes three species from western Europe (major anatomical references on which are: Wagner, 1915; Hesse, 1923; Künkel, 1933; Forcart, 1956; Giusti & Mazzini, 1971; Schileyko, 1986; Hausdorf, 2002; Nardi et al., 2007) and has unresolved relationships with *Semilimax* and ((*Semilimacella*, *Vitrina*) (*Eucobresia* (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*))))).

Apart from the type species, *Vitrinobrachium* includes only two species: *V. baccettii* from the Ligurian and Tuscan Apennines and Apuan Alps (Italy) and *V. tridentinum* Forcart, 1956, from Trentino - Alto Adige (Italy) (anatomy: Forcart, 1956; Nardi et al., 2007). Populations which differ considerably from typical specimens are assigned to these species, but they may be distinct taxa.

The species of *Vitrinobrachium* form a well-distinguished group, sharing many distinctive characters: penis with internal, glans-like structure, flat and divided into two parts; stimulator penial (erroneously considered atrial by Schileyko, 1986, and Hausdorf, 2002; vaginal by Muratov, 1999) in form of a club-like appendix (*V. breve* and *V. tridentinum*) or fused and englobed in distal part of penis (*V. baccettii*) with internal pedunculate sucker-like structure; vagina absent; duct of bursa copulatrix entering distal penis; penial retractor arising from columellar muscle.

All analyses found that *Vitrinobrachium* is monophyletic and supported by numerous synapomorphies (Figs. 14, 15; Table 3), although its deep relationships remain unresolved.

Vitrinids without Vaginal or Diverticular Stimulator

Vitrinids without vaginal or diverticular stimulator include three taxa of the genus group (*Calidivitrina*, *Eucobresia* and *Vitrina*), though other species with similar features belong to other genera, usually having the glandula amatoria (*Azorivitrina brevispira* and "*Madeirovitrina*" *media*). Our phylogenetic analyses suggested that they are derived taxa, which arose independently by loss of vaginal or diverticular stimulators.

Calidivitrina Pilsbry, 1919

Type Species: *Vitrina oleosa* Martens, 1895, by original designation.

Material Examined

Calidivitrina cf. *oleosa* (Martens, 1895) – Kenya: Cherangani near Kitale, 3000 m, A. Jeannel leg. 10.3.1933 (2 spirit specimens consisting of one already dissected adult and one intact juvenile, MNHN).

Diagnosis

Semislug-like vitrinids characterized by (short?) penial retractor muscle arising from diaphragm, compelling penial complex below right ommatophore and in front of right optic nerve and ending on penial complex subapically (at least in juveniles); stimulator absent; penial complex with penial gland and without penial sheath; vas deferens entering penial complex proximally; internal structures of penial complex comprising only main pilaster, consisting of large, distally lamellate, glandular roll and smooth slender distal muscular pleat.

Description of Type Species

Shell: vitriniform typical, glossy, thin and fragile, depressed, with approximately 3.0 whorls; last whorl expanded; aperture moderately wide; border of columellar and basal margins unknown; columellar margin normal; umbilicus closed; protoconch not prominent, smooth, without spiral rows of pits; teleoconch smooth (Pilsbry, 1919: 282, fig. 143a, b).

Body: available data uncertain (Martens, 1895; Pilsbry, 1919; Verdcourt, 2005). According to Pilsbry (1919: 283), shell lobes absent or, if present, very small. However Verdcourt (2005: 150, 152) stated that shell lobes are very well developed in most *Calidivitrina* species and sometimes cover whole shell.

Radula: dichoglossan; marginal teeth unicuspid (Pilsbry, 1919: 283; Hubendick, 1953: 89).

Female Distal Genitalia (Figs. 88–90): free oviduct short and wide. Bursa copulatrix medium in size and globular, with short, slender duct, initially not flared. Vagina short, without glandula amatoria, but with internal proximal pad. Internal surface of distal vagina with thin pleats, variably arranged.

Male Distal Genitalia (Figs. 88–90): penial complex large and club-like, with penial gland and without penial sheath. Penial gland covering most of proximal penial complex. Penial retractor arising from diaphragm, (short?), inserted apically. Vas deferens medium in length, entering penial complex proximally, thus distinguishing short pointed flagellum and penis proper. Internal structures of penial complex comprising main pilaster only which consists of large proximal glandular roll, distally lamellate, and slender smooth distal muscular pleat, ending in proximal genital atrium. Vas deferens opening not visible, probably level with glandular roll. Internal surface with thin irregularly arranged pleats or smooth.

Genital Atrium (Figs. 88–90): very long and cylindrical, with one side connected to body wall by thin muscular bands. Internal surface as in penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: unknown in adults but juveniles with entire penial complex below right ommatophore and in front of right optic nerve; right ommatophore is consequently shifted to right and compelled to lie between penial complex and vagina.

Mating: no data available.

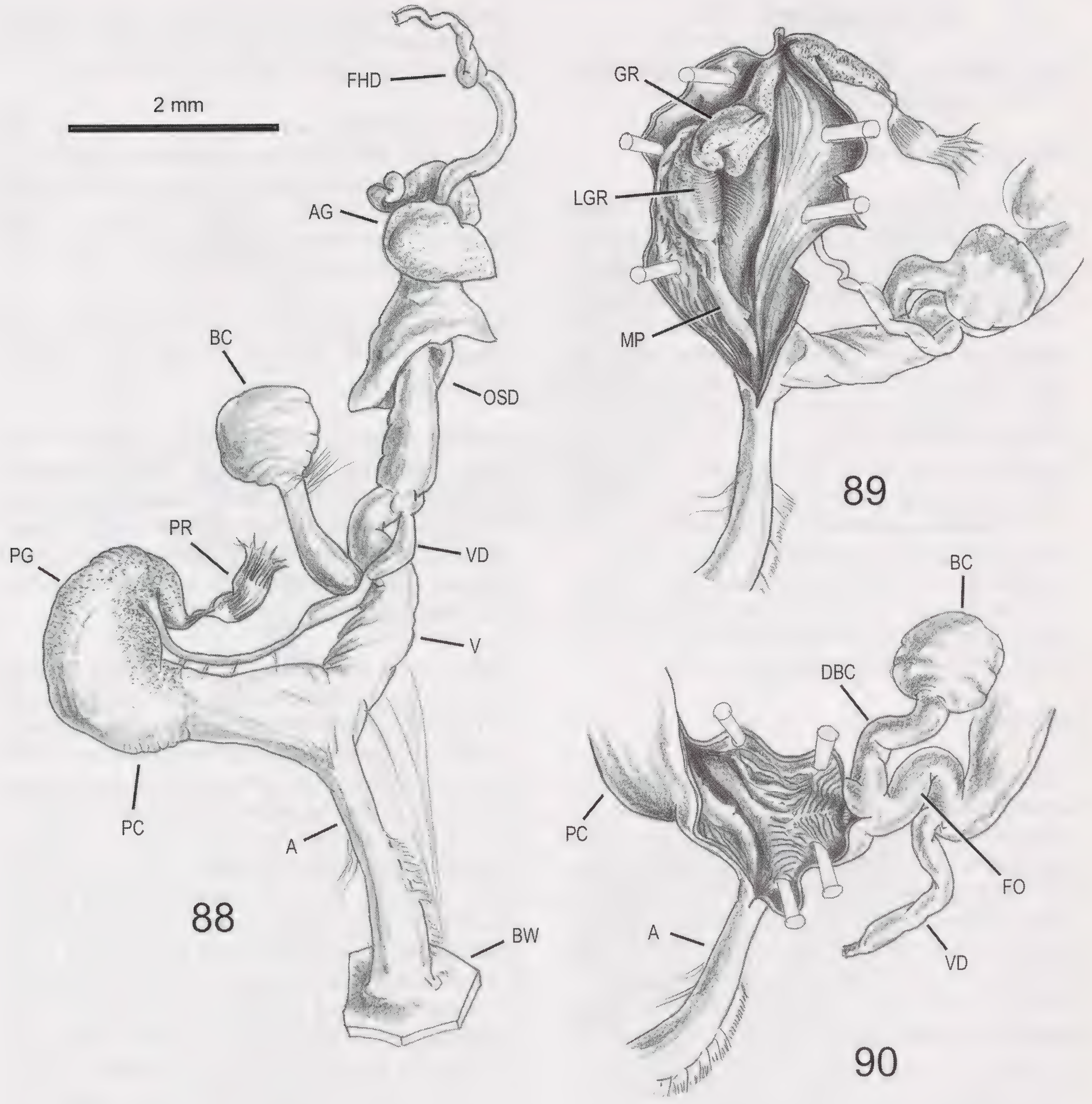
Remarks

Calidivitrina was established by Pilsbry (1919) as a new subgenus of *Vitrina*. Later it was considered a section of the subgenus *Vitrina (Vitrina)* by Thiele (1931), a junior synonym of *Vitrina (Vitrina)* by Hubendick (1953), a subgenus of *Semilimax* by Zilch (1959), a subgenus of *Vitrina* by Verdcourt (2005) and a distinct genus by Forcart (1978), Schileyko (1986) and Hausdorf (2002).

Hausdorf (2002) assigned eight species from Abyssinia, Kenya and Uganda to *Calidivitrina* (major anatomical references on which are: Hubendick, 1953; Forcart, 1957; Hausdorf, 2002; Schileyko, 2003) and regarded it as the sister group of *Arabivitrina*, and *Arabivitrina* plus *Calidivitrina* in turn the sister group of *Plutonia* plus *Oligolimax*.

The specimens dissected by us differ in some details with respect to those studied by Pilsbry (1919: 282–283, fig. 144c). They may therefore have been misidentified. However they were the only material available for our study.

Species assigned to *Calidivitrina* are relatively unknown because they have only



FIGS. 88–90. *Calidivitrina* cf. *oleosa* (Martens, 1895) from Cherangani near Kitale (Kenya), A. Jeannel leg. 10.3.1933. FIG. 88: Distal genitalia; FIG. 89: Internal structure of penial complex; FIG. 90: Vagina.

been summarily investigated, and there is no evidence that they constitute a monophyletic group. *Calidivitrina* includes Afrotropical vitrinids lacking a stimulator (glandula amatoria and diverticular stimulator), a feature shared by species such as *Eucobresia diaphana*, *Vitrina pellucida*, the Macaronesian *"Madeirovitrina" media* (Lowe, 1854) (cf. Groh & Hemmen, 1986) and *Azorivitrina brevispira* (Figs. 23, 24). *Vitrina pellucida* is distinct from all the others by virtue of different relationships between the right ommatophore retractor and the distal genitalia. *Arabivitrina brevispira* belongs to a genus endemic to the Azores on the basis of synapomorphic structure of the main pilaster. AP suggested that loss of the glandula amatoria happened at different times in different groups of vitrinids and that *Calidivitrina* belongs to a monophyletic clade together with *Guerrina* and *Madeirovitrina.* However, this clade must be considered with prudence because it is characterized by one non exclusive synapomorphy and may be biased by the fact that it was only possible to examine one species of *Calidivitrina* (two specimens: one adult already dissected and one juvenile).

Eucobresia Baker, 1929

Type Species: *Vitrina diaphana* Draparnaud, 1805, by original designation.

Material Examined

Eucobresia diaphana (Draparnaud, 1805) – Italy: Valtellina (Sondrio), 10.10.1952 (2 spirit specimens, FGC). Germany: Rheininsel Nonnenwerth (North Rhine-Westphalia), W. R. Schlickum leg. 17.11.1948 (2 spirit specimens, SMF 276318). Austria: Bregenzer Wald Damüls, Uga-Koft (Vorarlberg), H. Schönmann leg. 30.7.1973 (2 spirit specimens, NHMW 74317).

Eucobresia glacialis (Forbes, 1837) – Italy: Caverna della Gran Frana (Oulx, Torino), 32TLQ2893, S. Birindilli, E. Gavetti & E. Lana leg. 16.8.2000 (3 spirit specimens, FGC).

Eucobresia nivalis (Dumont & Mortillet, 1852) – Italy: Val Sesia, Col d'Olen (Alagna Valsesia, Vercelli), 32TMR1280, M. Bodon leg. 21.7.1990 (2 spirit specimens, FGC).

Diagnosis

Semislug-like vitrinids characterized by short penial retractor muscle arising from diaphragm, compelling penial complex below right ommatophore and in front of right optic nerve and ending on penial complex subapically (but juveniles with long penial retractor muscle running in front of right optic nerve, below right ommatophore retractor); stimulator absent; penial complex with penial gland and penial sheath; vas deferens contacting penial complex medially, traversing penial sheath, running along penial complex and entering it subapically; internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters; larger pilaster (main pilaster?) consisting of presumably glandular, proximal portion and low, robust, medial-distal muscular pleat ending in genital atrium, having two transverse rows of fused thorn-like structures on one side of larger pilaster, at about half its length.

Description of Type Species

Shell: vitriniform very dilated, glossy, thin and fragile, depressed, with approximately 2.5 whorls; last whorl extremely expanded; aperture very wide with columellar and basal margins bordered by broad periostracal fringe; columellar margin slightly cut-away; umbilical area open; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 7, fig. 1a–c).

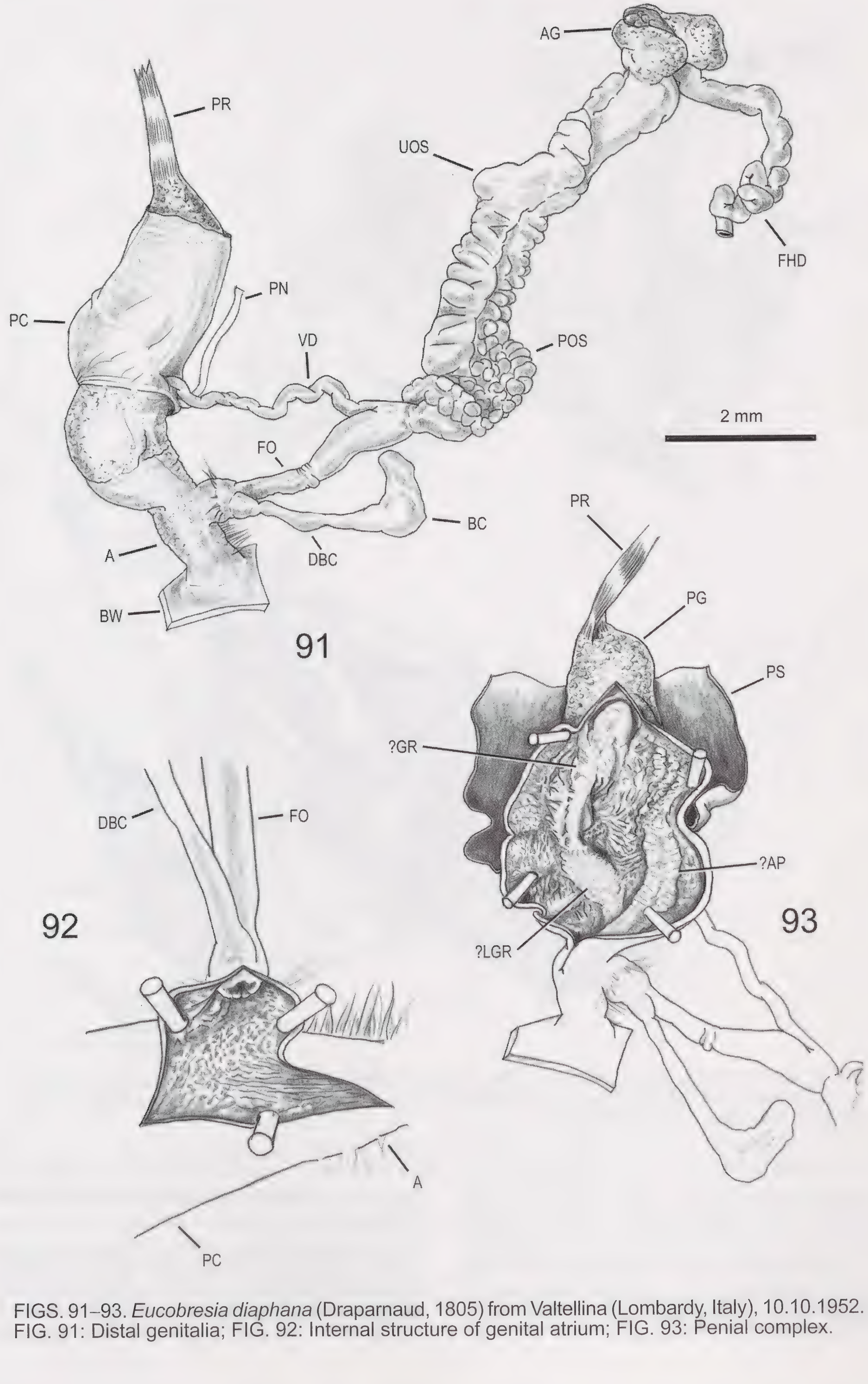
Body: cannot be withdrawn completely into shell; right shell lobe large, covering wide portion of shell when animal undisturbed (Kerney & Cameron, 1979: pl. 7, fig. 1d; Bogon, 1990: fig. at p. 183; Falkner, 1990: fig. 5 at p. 173; 1992: figs. 6–8 at p. 270).

Radula: dichoglossan; marginal teeth unicuspid (Wagner, 1915: 470, pl. 13, fig. 107; Hesse, 1923: 88).

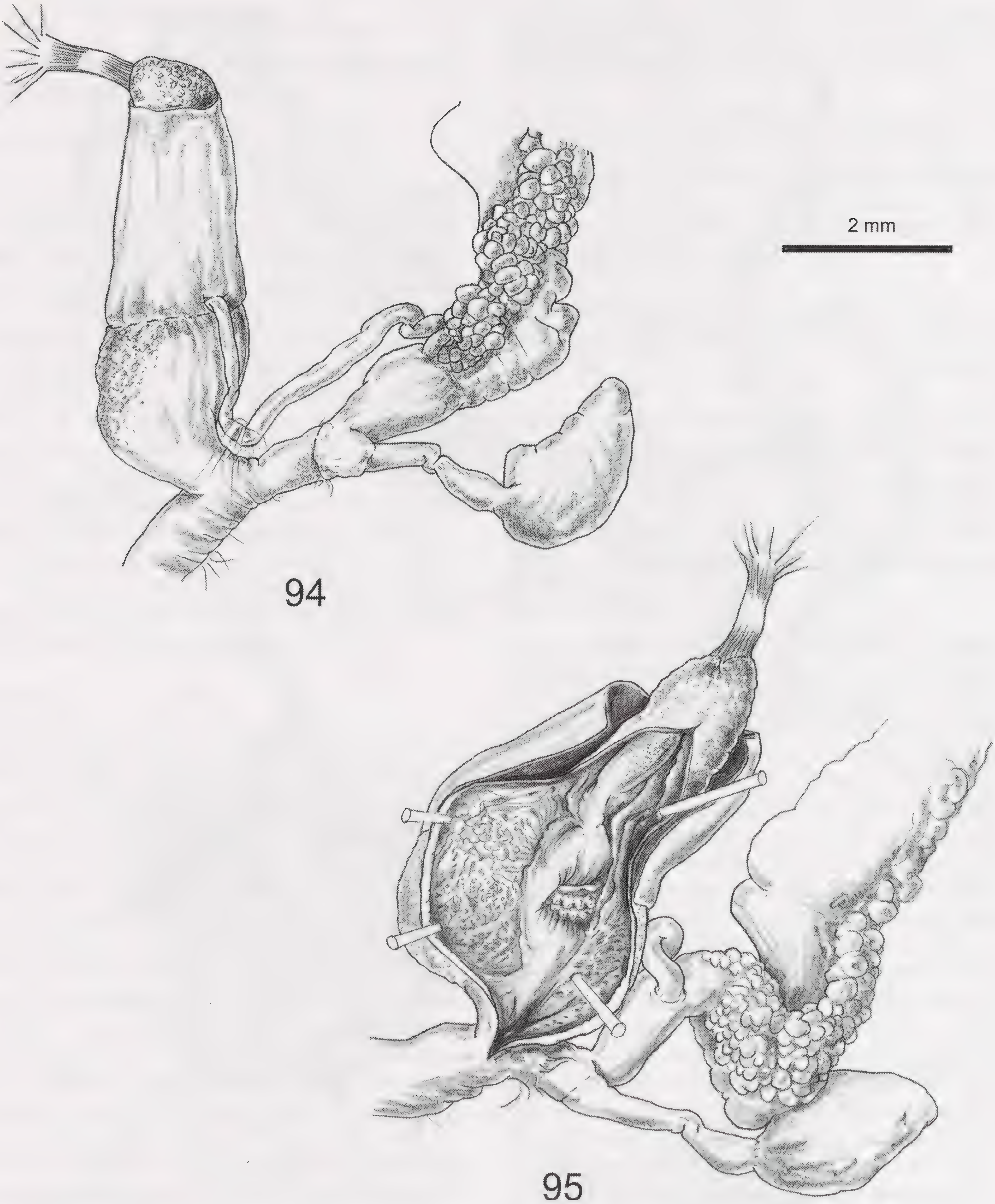
Female Distal Genitalia (Figs. 91–95): free oviduct short to long and wide. Bursa copulatrix small and oval to shoe-like in shape, with short to medium, more or less slender duct, initially not or only slightly flared. Vagina very short, without glandula amatoria but with internal proximal pad.

Male Distal Genitalia (Figs. 91–95): penial complex large and club-like, with penial gland and penial sheath. Penial gland covering most of penial complex. Penial sheath long, arising from mid penial complex and enveloping almost all proximal penial complex. Penial retractor arising from diaphragm, short, inserted apically. Vas deferens long (?), contacting penial complex medially, then traversing penial sheath, running along surface of penial gland and entering penial complex subapically near insertion of penial retractor. Internal structures of penial complex comprising two distinctive pilasters, possibly homologous with main and accessory pilasters. Larger pilaster (main pilaster?) consisting of presumably glandular proximal portion (modified glandular roll?) and low robust medial–distal muscular pleat ending in genital atrium (in one case muscular pleat gently transversely pleated before its final portion). One side of larger pilaster bearing two transversal rows of fused thorn-like structures at about half its length. Smaller pilaster (accessory pilaster?) arising level with medial portion of larger pilaster and ending in genital atrium. Vas deferens opening not visible, level with proximal penial complex, hidden by glandular tissue. Internal surface with thin irregularly arranged pleats and scattered spongy tissue with crypts.

Genital Atrium (Figs. 91–95): short, with one side connected to body wall by thin muscular bands. Internal surface as in penial complex.

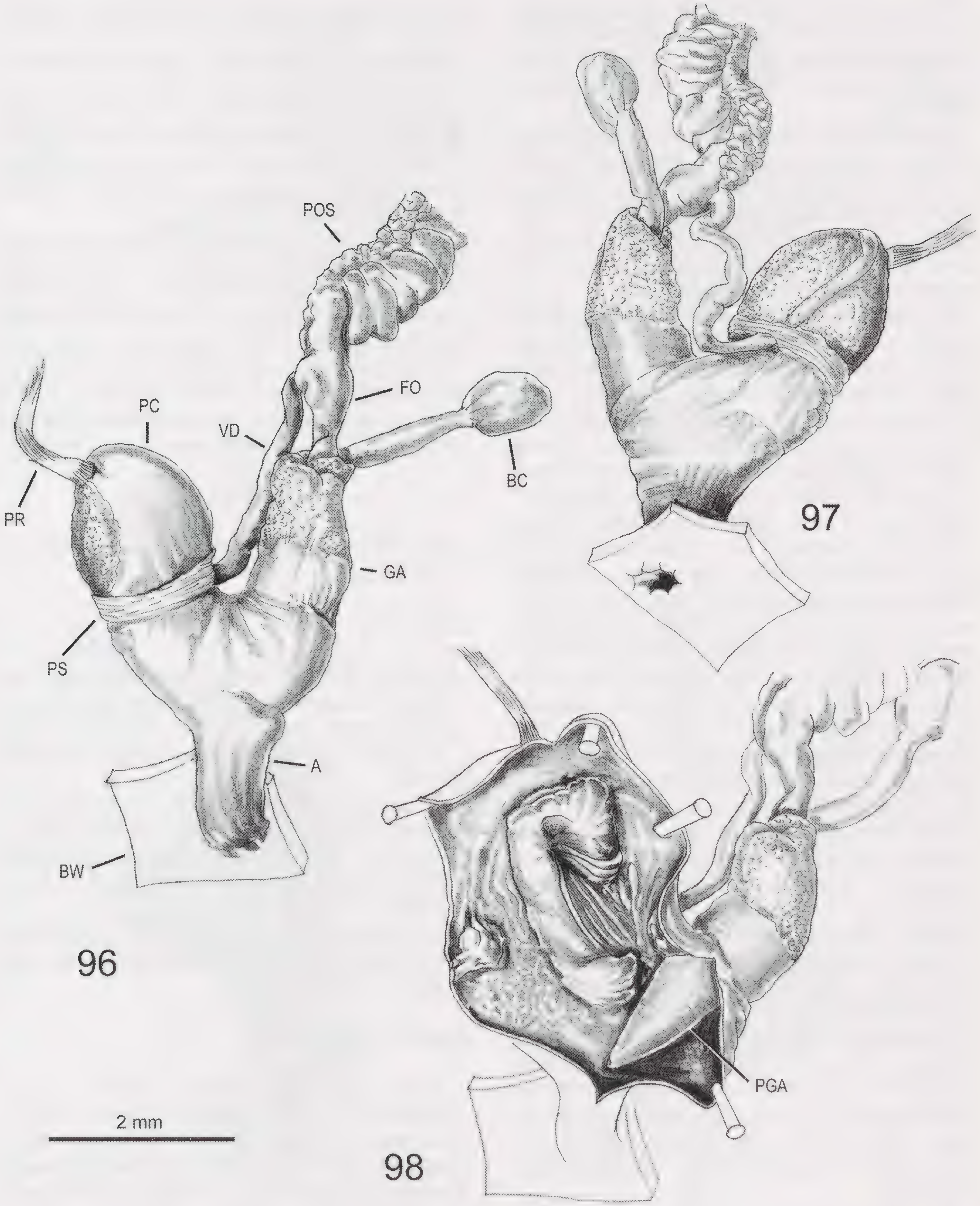


FIGS. 91–93. *Eucobresia diaphana* (Draparnaud, 1805) from Valtellina (Lombardy, Italy), 10.10.1952. FIG. 91: Distal genitalia; FIG. 92: Internal structure of genital atrium; FIG. 93: Penial complex.



FIGS. 94, 95. *Eucobresia diaphana* (Draparnaud, 1805) from Rheininsel Nonnenwerth (North Rhine-Westphalia, Germany), W. R. Schlickum leg. 17.11.1948. FIG. 94: Distal genitalia; FIG. 95: Internal structure of penial complex.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: age-dependent (Fig. 5). Juveniles with long slender penial retractor passing below right ommatophore and in front of right optic nerve; distal genitalia lying right of right ommatophore retractor which therefore appears free of penial complex and vagina. Adults with penial retractor short so that penial complex more or less is below right ommatophore and in front of right optic nerve; right ommatophore is consequently shifted right and compelled to lie between penial complex and vagina.



FIGS. 96–98. *Eucobresia glacialis* (Forbes, 1837) from Caverna della Gran Frana (Piedmont, Italy), S. Birindilli, E. Gavetti & E. Lana leg. 16.8.2000. FIGS. 96, 97: Distal genitalia; FIG. 98: Internal structure of penial complex and distal vagina.

Mating: described by Gerhardt (1936). Stimulator is not used for fixing partner and penis is inserted into vagina or duct of bursa copulatrix during copulation.

Remarks

Eucobresia was introduced by Baker (1929) as a new name for *Semilimax sensu* Hesse, 1923 (*non* Stabile, 1859). Later, it was regarded as a section of the subgenus *Vitrina (Vitrina)* and subsequently as a distinct genus by all authors (Forcart, 1944; Zilch, 1959; Schileyko, 1986; etc.).

According to Hausdorf (2002), *Eucobresia* includes four species from the Alps, Balkan Peninsula, central Europe and western Europe (major anatomical references: Forcart, 1944; Schileyko, 1986; Hausdorf, 2002) and is the sister group of (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*))).

Eucobresia diaphana has a very short, simple vagina without glandula amatoria but with a simple internal proximal pad, matching that described by Schileyko (1986: fig. 12B). On the contrary, Hausdorf (2002) stated that *Eucobresia* had a glandula amatoria without external glandular layer (which he considered an autapomorphy of this genus; Hausdorf, 2002: note 2 at p. 358). It is evident that he based this statement not on the type species but on another species (namely *E. glacialis*), which he supposed to belong to *Eucobresia* because it was characterized by the penial sheath and the right ommatophore retractor between penial complex and vagina. This species was redescribed by Forcart (1944) and assigned by him to *Insulivitrina* on the basis of the right ommatophore retractor being free of the distal genitalia, but Hausdorf (2002: note 2 at p. 358) sustained that Forcart was wrong.

According to Hausdorf (2002), *Eucobresia* included two other species, *E. nivalis* and *E. pegorarii* (Pollonera, 1884), besides the type species and *E. glacialis*. Unfortunately, the identity of the species assigned to *Eucobresia* is problematical because revisions have been performed without considering typical or topotypical material, and their relationships are still uncertain pending meticolous anatomical studies. For example, in disagreement with Hausdorf, *E. glacialis* has a glandula amatoria with external glandular covering, albeit incomplete (Figs. 96–98). We therefore limited the diagnosis of the genus to the characters of *E. diaphana*.

The other species included in *Eucobresia*, as they are currently conceived, have shells with columellar margin not cut-away and lack the two transverse crests of fused thorn-like structures inside the penial complex (these characters may be only species-specific: shells with columellar margin cut-away are found in other vitrinids, such as some Macaronesian species and some *Semilimax*). Apart from *E. glacialis*, the other species are characterized by progressive reduction of muscular section of glandula amatoria to an annular pad. For example, *E. nivalis* has a general scheme of the genitalia rather similar to that of *E. diaphana* but is distinguished by the fact that the vagina contains a rather well developed conical papilla which resembles that of a typical glandula amatoria, although there is no glandula amatoria (Figs. 99–102).

The vaginal pad of *E. diaphana* may be an extreme stage of reduction of the glandula amatoria of a *Phenacolimax*-like ancestor that went through stages exemplified by *E. glacialis* and then *E. nivalis*. Although we investigated this hypothesis considering three species of *Eucobresia* and two of *Phenacolimax* in the phylogenetic analysis, a clade including all five or at least some of them was not recovered.

Vitrina Draparnaud, 1801

Type Species: *Helix pellucida* Müller, 1774, by monotypy.

Synonyms

Cobresia Hübner, 1810. Type Species: *Cobresia vitrea* Hübner, 1810, by subsequent designation (Baker, 1929). *Cobresia vitrea* is a junior synonym of *Helix pellucida* Müller, 1774.

Helicolimax Férussac, 1801. Type Species: *Helix pellucida* Müller, 1774, by monotypy.

? *Hyalina* Studer, 1820, *non* Schumacher, 1817. Type designation not traced (Barker, 1999).

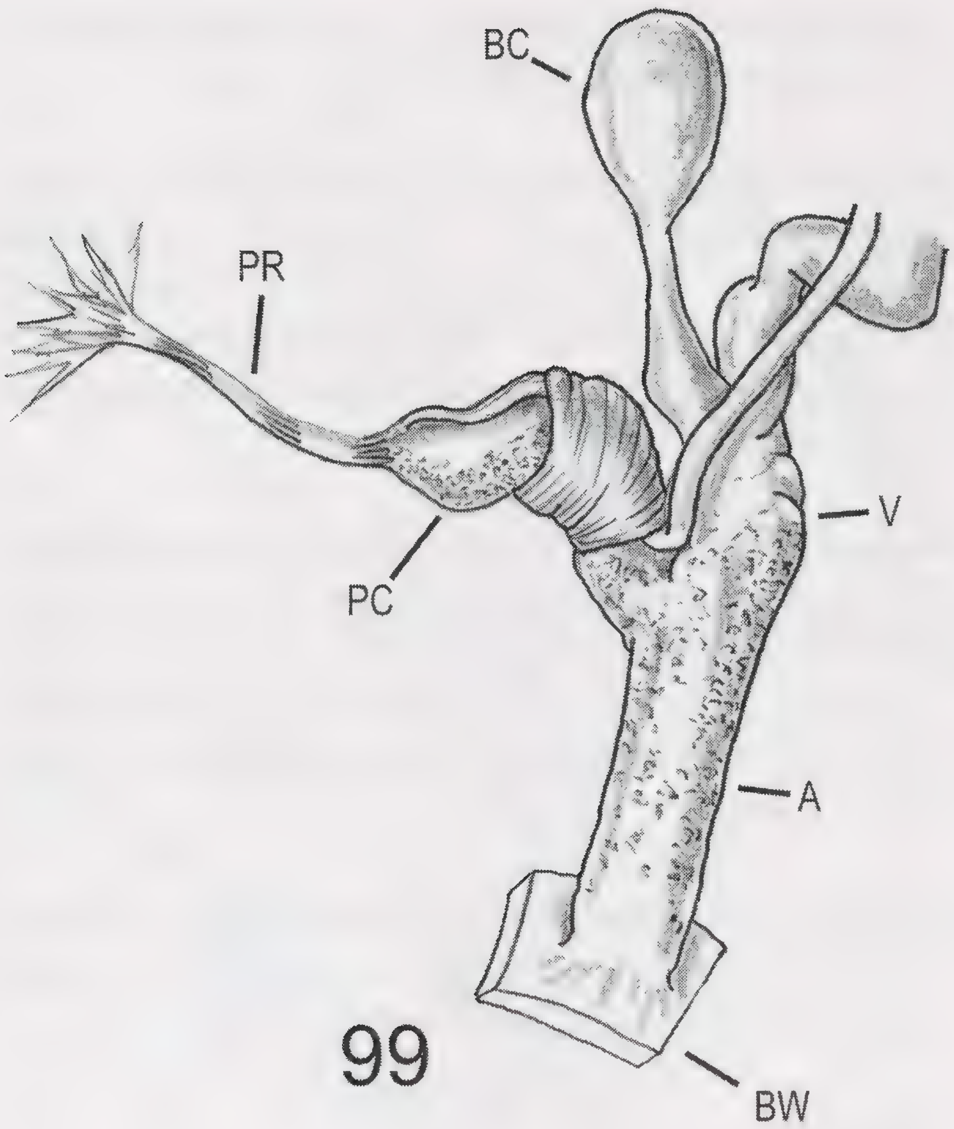
Pagana Gistl, 1847. Type Species: *Helix pellucida* Müller, 1774, by subsequent designation (Baker, 1929).

Material Examined

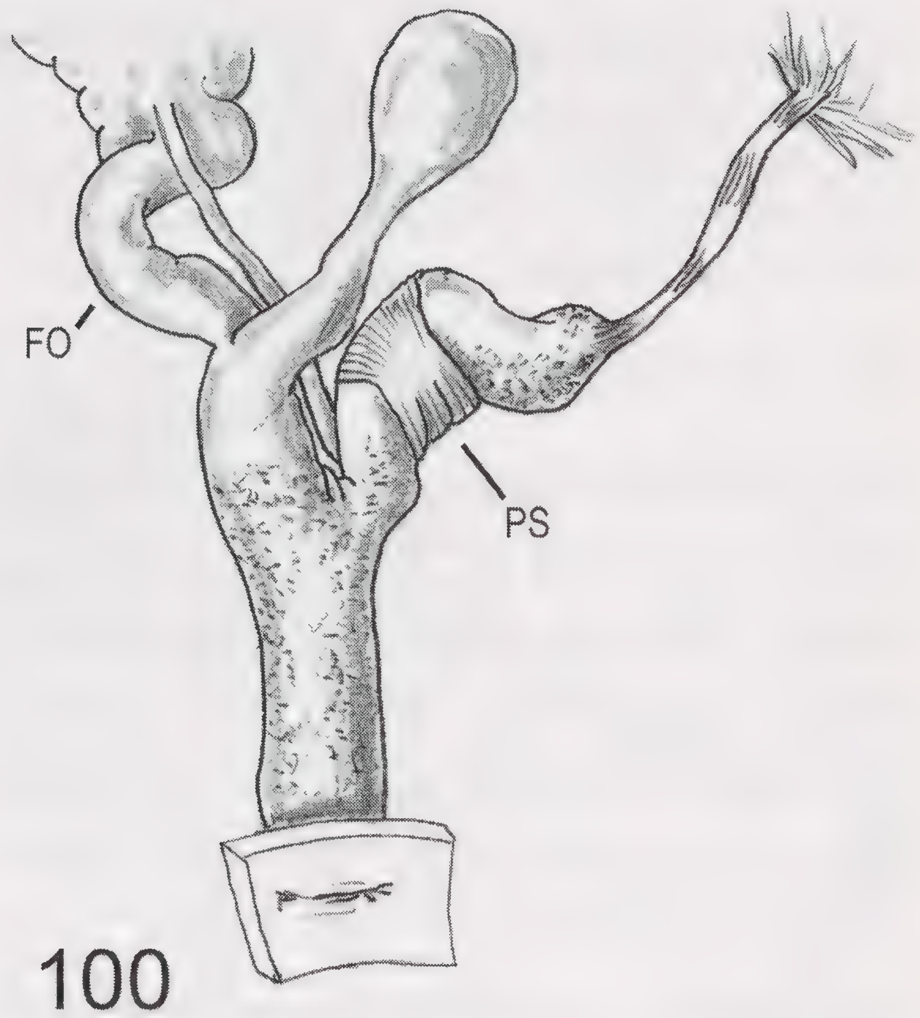
Vitrina pellucida (Müller, 1774) – Italy: Gasperone (Vernio, Prato), 32TPP7185, S. Cianfanelli & E. Lori leg. 30.10.2002 (1 spirit specimen, FGC). Bossea (Frabosa Soprana, Cuneo), M. Bodon leg. 9.4.1987 (1 spirit specimen, FGC). United Kingdom: Dollar Glen (Scotland), F. Giusti & G. Manganelli leg. 3.9.1986 (5 spirit specimens, FGC).

Diagnosis

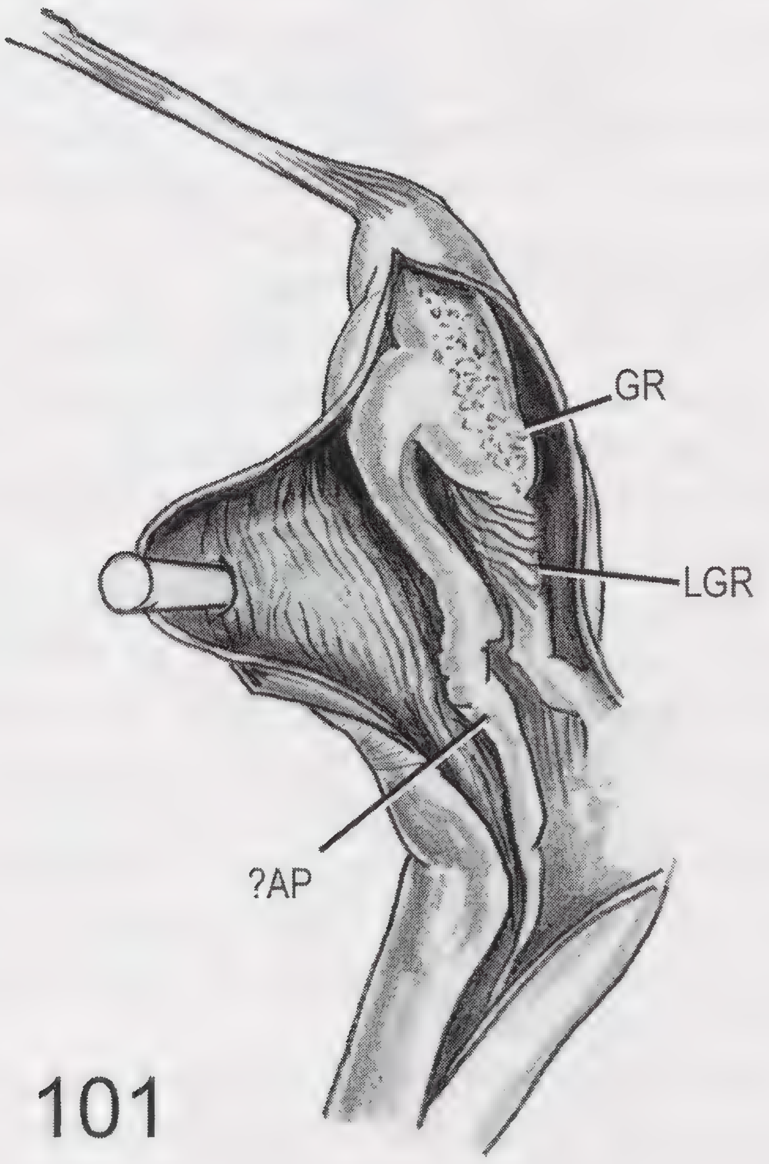
Semislug-like vitrinids characterized by long penial retractor muscle arising from diaphragm, passing above right ommatophore retractor and ending on penial complex subapically; stimula-



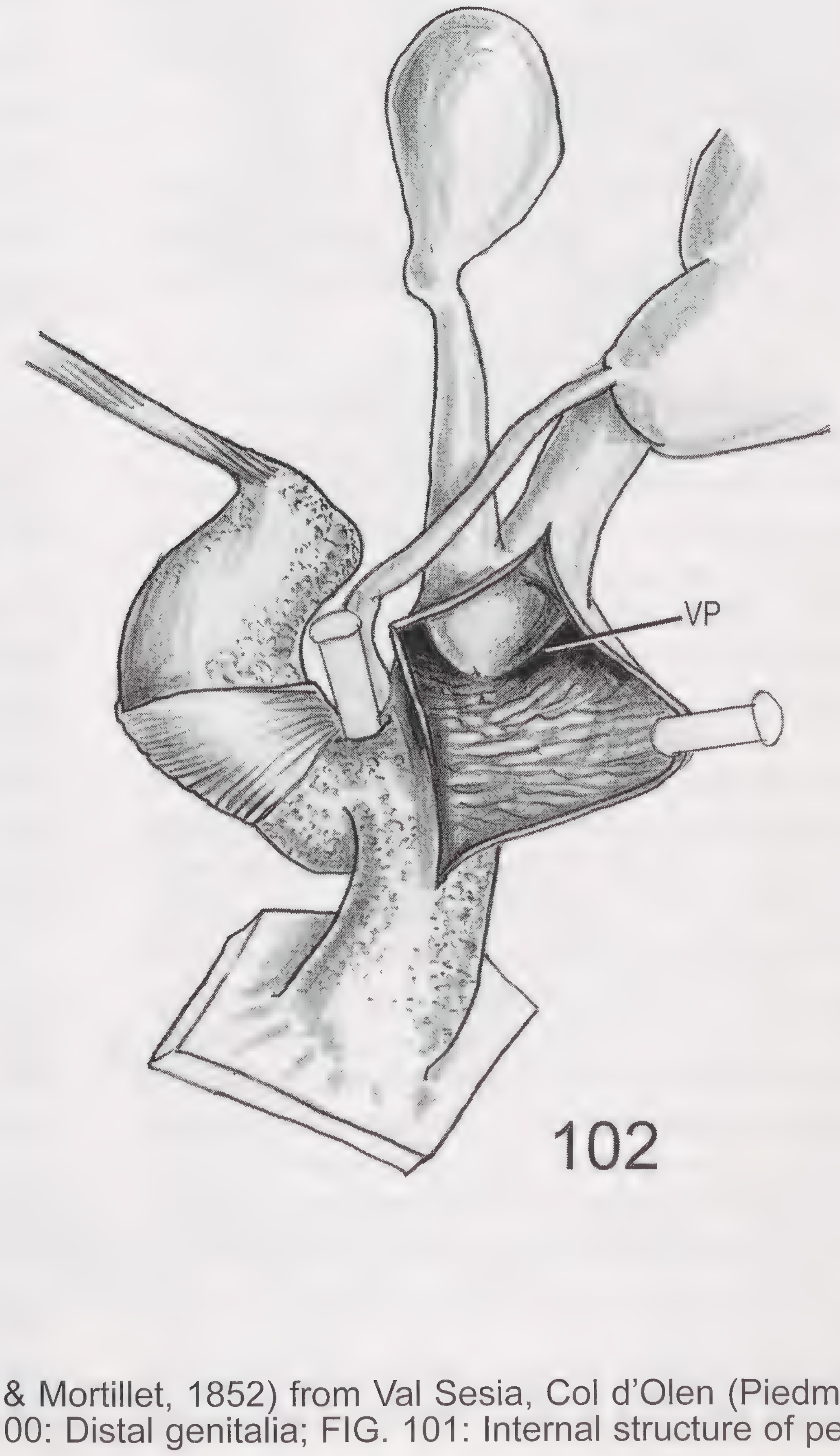
99



100



101



102

99, 100 2 mm
101, 102 1 mm

FIGS. 99–102. *Eucobresia nivalis* (Dumont & Mortillet, 1852) from Val Sesia, Col d'Olen (Piedmont, Italy), M. Bodon leg. 21.7.1990. FIGS. 99, 100: Distal genitalia; FIG. 101: Internal structure of penial complex; FIG. 102: Distal vagina.

tor absent; penial complex with penial gland and penial sheath; vas deferens contacting penial complex distally, traversing penial sheath, running along penial complex and entering it apically; internal structures of penial complex comprising only main pilaster consisting of slender, not distally lamellate, glandular roll.

Description of Type Species

Shell: vitriniform typical, glossy, thin and fragile, globular-depressed, with approximately 3.0 whorls; last whorl expanded; aperture moderately wide with basal margin bordered by narrow periostracal fringe; columellar margin normal; umbilicus open, punctiform; protoconch not prominent, smooth, with many regular spiral rows of very small pits; teleoconch smooth (Kerney & Cameron, 1979: pl. 6, fig. 1a–c).

Body: cannot be withdrawn completely into shell; right shell lobe small, covering very limited portion of shell when animal undisturbed (Kerney & Cameron, 1979: pl. 6, fig. 1d; Falkner, 1990: fig. 1 at p. 173).

Radula: dichoglossan; marginal teeth pluricuspid (Taylor, 1906: 6, figs. 8, 9; Wagner, 1915: 471, pl. 13, fig. 95; Castillejo, 1985: 66, fig. 1).

Female Distal Genitalia (Figs. 103–105): free oviduct short and wide. Bursa copulatrix medium in size and sac-like with short, more or less slender duct, initially very flared. Vagina very short, without glandula amatoria or other internal structures, but with external glandular covering also on initial part of duct of bursa copulatrix.

Male Distal Genitalia (Figs. 103–105): penial complex small and finger-like, with penial gland and penial sheath. Penial gland covering most of penial complex. Penial sheath long, sometimes curled up, arising from end of penial complex and enveloping almost all penial complex. Penial retractor arising from diaphragm, long, inserted apically. Vas deferens long, contacting penial complex distally, then traversing penial sheath, running along surface of penial gland and entering penial complex apically near insertion of penial retractor. Internal structures of penial complex comprising main pilaster only, which consists of slender glandular roll not distally lamellate, ending before genital atrium. Vas deferens opening not visible, probably level with initial part of glandular roll. Internal surface with sparse spongy tissue with crypts.

Genital Atrium (Figs. 103–105): long to very long and cylindrical, with sparse glandular covering (in close continuity with that on vagina) and opposite sides connected to body wall by thin muscular bands. Internal surface with low pleats.

Relationships Between Right Ommatophore Retractor and Distal Genitalia: similar in juveniles and adults (Fig. 10). Penial retractor long, passing above right ommatophore retractor which is consequently free of penial complex and vagina.

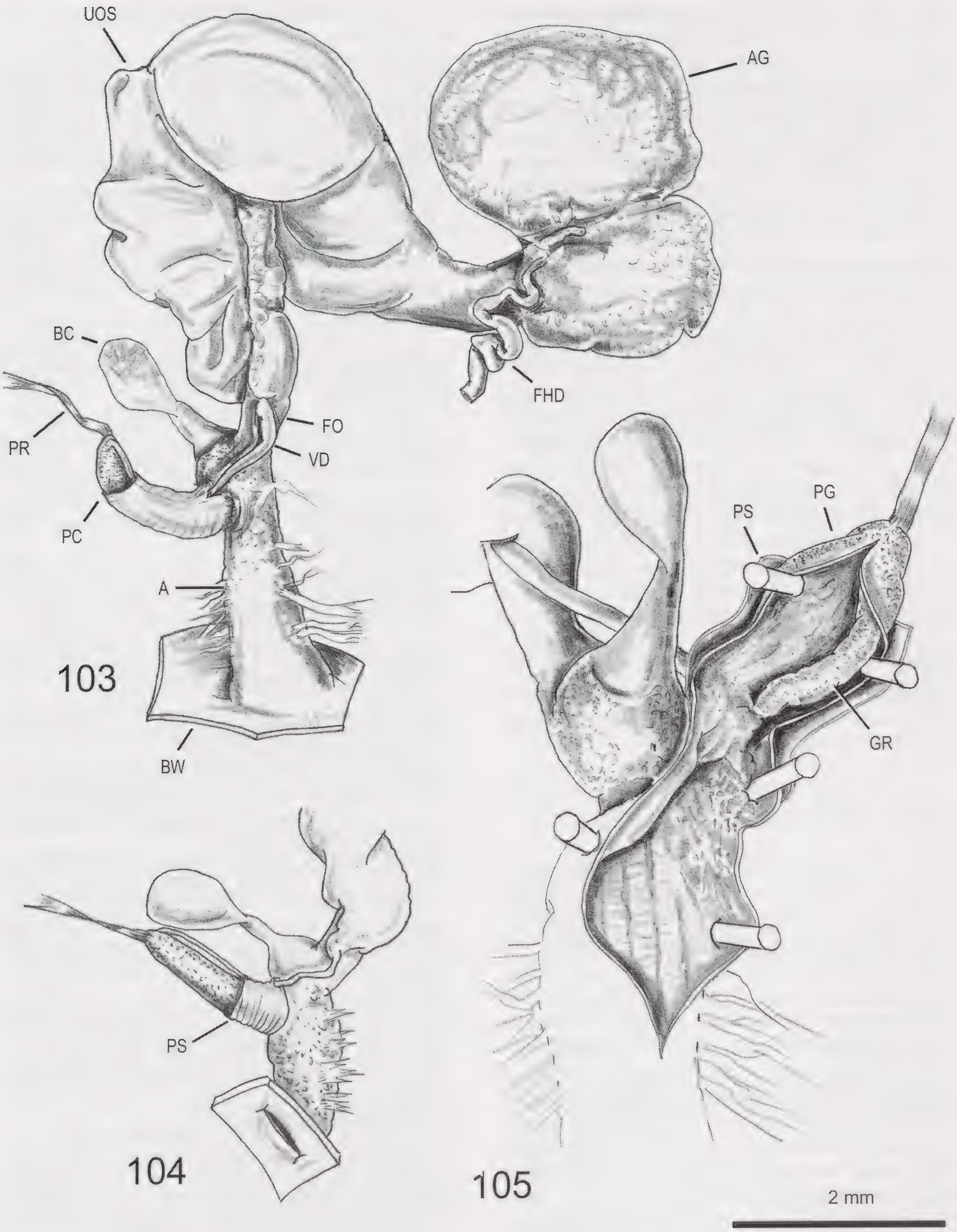
Mating: Hausdorf (2002) stated that in *Vitrina*, penis is inserted in vagina or duct of bursa copulatrix during copulation.

Remarks

Vitrina is the first genus-group taxon established for this group of land pulmonates. The genus has Holoarctic distribution and according to Hausdorf (2002) includes only three anatomically known species (major anatomical references on which are: Eckardt, 1914 [not seen; fide Hausdorf (2002)]; Pilsbry, 1946; Forcart, 1955; Umiński, 1968; Schileyko, 1986, 2003) and is the sister group of *Semilimacella*; in turn *Semilimacella* plus *Vitrina* is sister group of (*Eucobresia* (*Phenacolimax* ((*Plutonia*, *Oligolimax*) (*Arabivitrina*, *Calidivitrina*)))).

In disagreement with Hausdorf (1995, 2002) and Schileyko (2003), we are of the same opinion as Lohmander (1938), that *Vitrina pellucida* has a vagina, though very short. Its presence is confirmed by the fact that in adult specimens its walls (like those of initial part of duct of bursa copulatrix) are covered by glandular tissue (like in species of *Semilimacella* and oxychiline zonitids).

The other anatomically known species are *V. angelicae* Beck, 1837, from Greenland and North America and *Vitrina rugulosa* Martens, 1874, from Asian Russia. Although these species share the same scheme of the genitalia, they apparently differ in important details and it is therefore unclear whether they constitute a monophyletic group together with *V. pellucida*. *V. angelicae* differs from *V. pellucida* by virtue of duct of bursa copulatrix initially not flared and absence of penial sheath (for anatomy: Soós & Schlesch, 1924; Forcart, 1955). *V. rugulosa* dif-



FIGS. 103–105. Distal genitalia of specimens of *Vitrina pellucida* (Müller, 1774). FIGS. 103, 105: Specimen from Gasperone (Tuscany, Italy), S. Cianfanelli & E. Lori leg. 30.10.2002; FIG. 104: Specimen from Bossea (Piedmont, Italy), M. Bodon leg. 9.4.1987.

fers from *V. pellucida* by virtue of duct of bursa copulatrix initially not flared and pad bordering opening of duct of bursa copulatrix into vagina (for anatomy: Schileyko, 1986).

AP (DPIII) suggested that *V. pellucida* forms a monophyletic group with *Semilimacella bonelli* (61% symmetric resampling, 0.22/67 absolute and relative Bremer supports) on the basis of at least one synapomorphy (bursa copulatrix duct initially very flared). Moreover, MP and AP showed that these two species belong to a larger monophyletic group having very low support (MP: 50%; AP: 9% symmetric resampling, 0.15/40 absolute and relative Bremer supports) including four other species (*Oligolimax annularis*, *O. musignani*, *Sardovitrina polloneriana* and *Semilimacella cephalonica*) based on exclusive disposition of penial retractor above right ommatophore retractor. Relationships between *V. pellucida* and the vitrinids with atrial-vaginal stimulator are not new. Indeed, Umiński (1968) described a small diverticulum located on the proximal atrium opposite the penial complex in juveniles of *Vitrina pellucida*. He compared it with the atrial stimulator of *Hessemilimax kotulae* and in the absence of definitive evidence about homology of the two structures, he concluded that the small atrial diverticulum of *Vitrina pellucida* is probably a vestige of an atrial stimulator.

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RESEARCH NOTES

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SURVIVAL OF THE INVASIVE CLAM *CORBICULA FLUMINEA* (MÜLLER) IN RESPONSE TO WINTER WATER TEMPERATURE

Oliver Müller & Bruno Baur*

Department of Environmental Sciences, Section of Conservation Biology, University of Basel,
St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland

ABSTRACT

Water temperature plays a crucial role in determining the distribution of aquatic organisms because most are ectothermic. The further spread of the invasive Asian clam *Corbicula fluminea*, a species causing enormous damage to water intake pipes and electric power plant cooling systems, has been assumed to be limited due to intolerance of very cold water, with a lower lethal temperature of about 2°C. In recent years, however, *C. fluminea* invaded most of the major rivers in Europe, including sections where the water temperature falls below 2°C for short periods during winter. Here we quantify the cold tolerance of *C. fluminea* in a controlled laboratory experiment. We show that *C. fluminea* has a greater cold tolerance than previously assumed. In water of 0°C, clam survival decreased from 100% to 17.5% with increasing exposure from 4 to 9 weeks. Considering actual water temperatures of rivers during winter and ongoing stream warming, we conclude that this invasive clam will establish in a wider range of waterbodies in Europe than previously predicted.

Key words: Asian clam, biological invasion, cold tolerance, *Corbicula fluminea*, winter survival.

INTRODUCTION

Freshwaters are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems (Dudgeon et al., 2006). Besides water pollution, destruction or degradation of habitat and flow modification, the invasion of non-native species is a major threat to native freshwater biodiversity (Strayer, 1999). In the past decades, European inland waters have been increasingly affected by the colonisation of alien species, causing significant changes in aquatic communities (Baur & Ringeis, 2002; Baur & Schmidlin, 2007). Nowadays, more than 95% of the biomass of benthic macroinvertebrates in the river Rhine consists of non-native species (Bij de Vaate et al., 2002; Wirth et al., 2010).

Originating from Southeast Asia, the freshwater clam *Corbicula fluminea* (Müller, 1774) has been introduced in North and South America and in Europe (McMahon, 1983; Beasley et al., 2003; Morgan et al., 2003; Karatayev et al., 2005; Schmidlin & Baur, 2007). The rapid spread and persistence of *C. fluminea* throughout Europe, North and South America is related to its rapid growth rate, early onset of maturity, high fecundity, and its ability to tolerate a wide range of environmental conditions (Mattice & Dye, 1976; McMahon, 1983, 2002). In the river Rhine, *C. fluminea* was first recorded in The Netherlands in 1985 (Glöer & Meier-Brook, 1998). Since then, the invasive clam is spreading in rivers and standing waters, reaching densities of up to 10,000 individuals per m^2 (in the River Rhine near Basel; Mürle et al., 2008). *Corbicula fluminea* clogs water intake pipes, electric power plant cooling systems and sewage treatment plants, causing enormous damage (Pimentel et al., 2007). In the U.S.A., costs associated with this clam are estimated to be more than $1 billion per year (OTA, 1993). As a dominant species of the macrozoobenthos, *C. fluminea* is sequestering a large proportion of the carbon available for benthic production and altering the ecosystem functioning (Sousa et al., 2008). The invasive clam decreases the abundance of benthic flagellates, bacteria and diatoms and affects other organisms by bioturbating sediments (Hakenkamp et al., 2001). Valves of dead *C. fluminea*, however, can increase the surface area and substratum diversity of sandy bottoms,

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*Corresponding author: bruno.baur@unibas.ch

resulting in an increase of benthic invertebrates (Werner & Rothhaupt, 2007).

The distribution of invasive freshwater species in novel areas is frequently determined by their tolerance to low and high water temperatures. The invasive *C. fluminea* has been widely perceived as limited in its range due to intolerance of cold water (McMahon, 1983). This reflects the relatively mild winter temperatures *C. fluminea* experiences in its native habitats in Southeast Asia. In a laboratory study of thermal tolerance, Mattice & Dye (1976) demonstrated that the incipient lower lethal temperature of *C. fluminea* was about 2°C. Since then, this lower lethal temperature has been cited repeatedly without further examination, even in the most recent *Handbook of Alien Species in Europe* (DAISIE, 2009). Using water temperature data of various rivers and considering 2°C as lower lethal temperature, Schöll (2000) predicted the spread of *C. fluminea* in Germany and concluded that without heat pollution, the species cannot establish in most rivers. In recent years, however, *C. fluminea* has spread in most of the major rivers in Europe, including sections where the water temperature falls below 2°C for short periods during winter (Schmidlin & Baur, 2007). Thus, the precise thermal tolerance of *C. fluminea* is not clear (Janech & Hunter, 1995), so it is impossible to predict its potential distribution range in Europe. Here, we present the results of a laboratory experiment designed to quantify the cold tolerance of *C. fluminea*.

MATERIALS AND METHODS

Corbicula fluminea is a simultaneously hermaphroditic filter- and pedal-feeding freshwater clam. It can reach sexual maturity at an age of 3–6 months (valve length of 6–10 mm) and reproduces by cross- and self-fertilization, releasing 300–400 pediveliger larvae of 200 µm length per day. In the River Rhine, *C. fluminea* is most abundant on fine-grained substrata (sand) with slowly flowing water (Schmidlin & Baur, 2007). Molecular analysis of a subunit of the mtCOI gene of *C. fluminea* revealed that all individuals collected in the rivers Rhine, Elbe and Upper Danube belong to the same haplotype (Stephanie Schmidlin, unpubl. data).

Specimens of *C. fluminea* were collected from the Altrhein, a remnant of the former river Rhine, 4 km downstream of Basel (47°37'29.7"N, 7°34'12.5"E) in March 2008. In the preceding three months these clams have been exposed to water temperatures ranging from 5.0 to 8.6°C. Prior to the experiment, the clams were acclimated to 8°C for seven days in containers filled with water from the River Rhine. We measured the size of each individual (maximum valve length) to the nearest 0.1 mm using a vernier calliper and assigned them to two groups: small clams (valve length 6.5–15.0 mm) and large clams (15.1–30.2 mm).

To examine the cold tolerance of *C. fluminea*, individuals were exposed to different water temperatures for periods of different lengths. The experimental design included the factors temperature (0°, 2° and 8°C), length of exposure (1, 2, 3, ..., 8, and 9 weeks) and size (small and large clams). Two replicates of 10 clams each were kept at each combination of factors (3 x 9 x 2) resulting in a total of 1,080 clams.

Following acclimation to 8°C for seven days, each ten small and ten large clams were placed in 10 x 11 x 9 cm compartments of plastic containers, whose bottoms were lined with 3 cm sand. Sand, sieved at a mesh size of 2 mm, was obtained from a site in the Rhine where *C. fluminea* occurred. Clams were submersed in water (6 cm) and the containers were kept in refrigerated incubators at constant temperatures of 0°, 2° and 8°C and darkness (clams are buried in sand during winter). To provide food (bacteria, phytoplankton) and oxygen, the water was exchanged every second day. Water from the River Rhine was cooled to the different experimental temperatures prior to exchange.

Every week we removed 20 clams (ten small and ten large individuals) per temperature treatment and replicate and tested their viability by forcing the tip of a dissection needle several millimeters between the posteriori valve margins in the region of the siphons (Matthews & McMahon, 1999). Living individuals strongly resist needle entry by tight clamping their valves. In contrast, dead clams offer little resistance and fail to close their valves after needle removal. The testing does not harm clams.

We used the R statistical computing software for data analysis (R Development Core Team, 2006). We applied a two-factorial ANOVA with repeated measurements to examine the effects of water temperature and duration of exposure on the survivorship of clams (Fig. 1). We used logistic regression to analyse any potential effect of individual clam size on survival. We built a generalised linear model (GLM) with a binomial error distribution and logit link (Fig. 2). The model consisted of the binary response variable (alive or dead) of each individual clam after a given exposure time and the independent categorical variable water temperature, and the two continuous variables length of exposure and individual clam size.

RESULTS

The water temperature significantly influenced the survival of overwintering *C. fluminea* ($F_{2.48}$ = 25.77, $p < 0.0001$; Fig. 1). Considering the entire experiment, 98.6% of the clams survived at 8°C, whereas 86.1% and 75.3% survived at 2° and 0°C, respectively. Furthermore, the length of exposure to cold water affected clam survival ($F_{1.48}$ = 99.86, $p < 0.0001$). Survival was constantly high at 8°C over the nine-week experiment. In water of 2° and 0°C, survival was high in the first four weeks, but decreased rapidly afterwards (interaction temperature x length of exposure: $F_{2.48}$ = 4.37, $p = 0.0181$; Fig. 1). Considering exclusively clams that were kept in cold water for 9 weeks, 47.5% survived at 2°C and 17.5% at 0°C.

Survival was also size-dependent ($F_{1.341}$ = 10.92, $p = 0.0011$; Fig. 2). In this analysis only clams that were exposed to different water temperatures for 7–9 weeks were considered. At 8°C, there was almost no mortality independent of valve size, whereas at 2°C and 0°C survival increased with valve size (Fig. 2). Larger clams had a higher probability to survive than smaller clams at water temperatures of 2° and 0°C (significant differences in the intercepts of the fitted lines, in each comparison $p < 0.001$). Survival was also size-dependent when all clams used in the experiment were considered ($F_{1.1025}$ = 9.73, $p = 0.0019$).

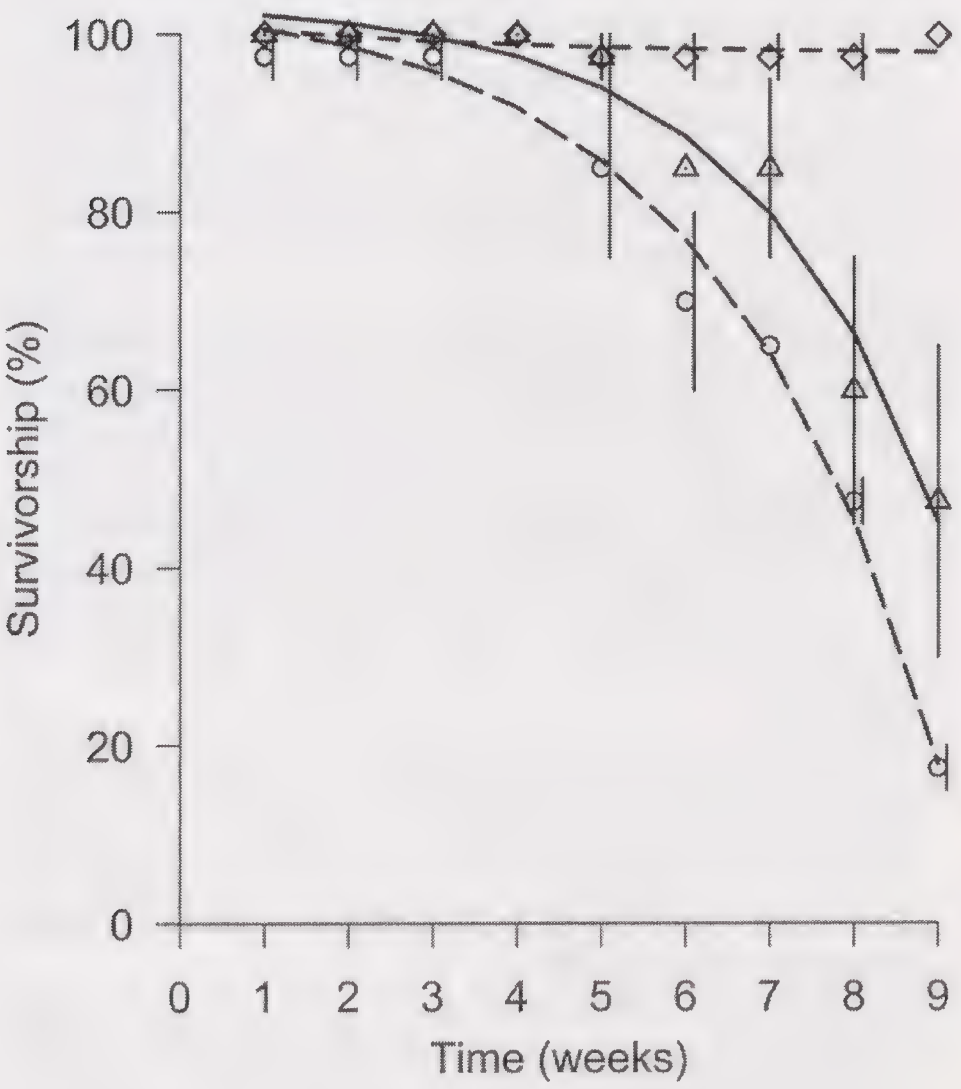

FIG. 1. Survival of *C. fluminea* exposed to water of different temperature (8°C, diamonds; 2°C, triangles; 0°C, open dots). Means (± 1 s.d.) of two replicates with 20 clams each are shown.

DISCUSSION

Our results show that *C. fluminea* has a much better cold tolerance than previously assumed. This could be explained by adaptation to cold water in temperate regions in the past decades, by phenotypic plasticity or by a non-optimal study of Mattice & Dye (1976) to determine the lower lethal temperature of this species. Janech & Hunter (1995) questioned the lower lethal temperature of 2°C because they recorded overwintering success of *C. fluminea* populations in North America despite water temperatures of 0–2°C for eight weeks. We also showed that clam survival in cold water decreased with exposure duration. In a ten-year field study in the Lower Connecticut river, New England, winter survival of *C. fluminea* was positively correlated with the average winter water temperature and negatively correlated with the frequency of daily mean water temperature ≤ 1°C (Morgan et al., 2003). In another study, condition indices decreased significantly in *C. fluminea* exposed to about 2°C for one month (French & Schloesser, 1996). Large individuals might have a better ability to store energy and therefore have a better resistance against different stressors than small clams. This could explain the size-dependent survivorship found in our study.

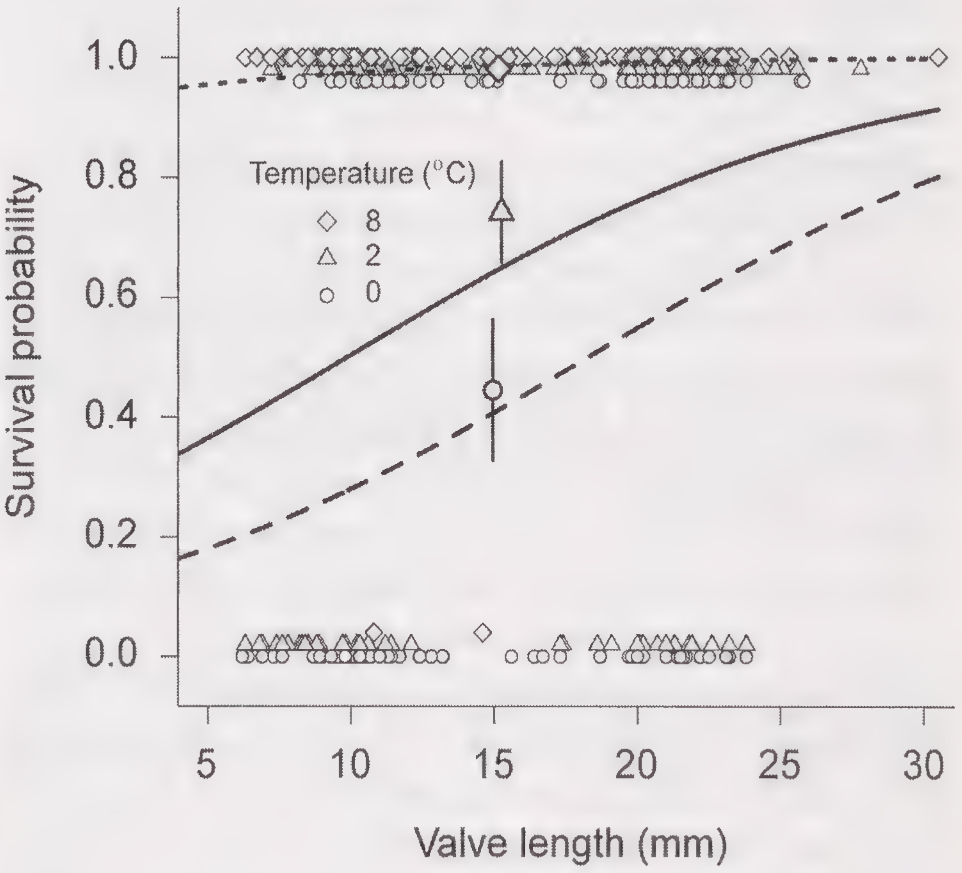

FIG. 2. Relationships between valve length of *C. fluminea* and the probability to survive in individuals exposed for 7–9 weeks to water of different temperature (lines show the model fit: 8°C, dotted line; 2°C, continuous line; 0°C, dashed line). Each symbol represents the status (alive or dead) of one individual. Mean survival probabilities and 95%-confidence intervals are also shown. At 8°C, there was almost no mortality independent of valve size, whereas at 2°C and 0°C survival increased with valve size.

The higher mortalities recorded at 2° and 0°C are most probably not a result of the abrupt temperature decrease at the beginning of the experiment following the pre-conditioning at 8°C, because clam survival was also high in the first four weeks at low water temperature. Our experiment simulated the conditions of an extended (and harsh) winter in a river fed with cold melt water. In most central European rivers, the water temperature falls below 2°C only for short periods of one to three weeks during winter. Our study shows that a significant portion of *C. fluminea* is able to survive short periods of cold water. However, when a period of extreme cold water exceeds two months, high mortality in *C. fluminea* may occur, as observed in Lake Constance in winter 2005/2006 (Werner & Rothhaupt, 2008).

The tolerance to low temperature may play a significant role in the future spread of *C. fluminea*. The length of cold water periods in rivers and lakes may limit the range expansion of this invasive clam. Eleven years (1997–2007) of daily water temperature data from 78 sites show that the minimum water temperature rarely falls below 2°C in most rivers in the Swiss lowlands (Müller, 2009). In a few rivers, the water temperature sank below 2°C for a period of 10–20 days in one to five years of the 11 years examined. During summer the water temperature of most rivers exceeds 16°C for at least one month which allows successful reproduction (Meister, 1997). In alpine rivers, however, the temperature requirements for clam survival and reproduction are not fulfilled. Niche modeling considering the temperature tolerance of the clam and extensive water temperature data revealed that almost all lowland rivers and lakes in Switzerland could be invaded by *C. fluminea* (Müller, 2009).

The spread of *C. fluminea* may be promoted by discharge of warmer industrial and residential water into natural waterways. In fact, downstream from cooling water outlets of power stations, populations of *C. fluminea* reach high densities (Morgan et al., 2003). Experimental warming in winter increased the clams' growth rate and enhanced the reproductive output (Weitere et al., 2009). From 1976 to 2000, mean air temperature at Basel and Zurich increased by 0.57°C per decade and stream temperature follows ambient air temperature closely (Hari et al., 2006). This means that global warming will facilitate the range expansion of invasive aquatic species (Heino et al., 2009). Our findings suggest that *C. fluminea* is able to establish in a much wider range of rivers and lakes than previously assumed.

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ON THE GENERIC ALLOCATION OF *"ALIGENA" PISUM* DALL, 1908

Marina Güller[1]* & Diego G. Zelaya[1, 2]

INTRODUCTION

Between 1887 and 1888, the United States *Albatross* conducted an expedition to the southern tip of South America. The bivalves from that expedition were studied by Dall (1889, 1890, 1896, 1901, 1902, 1908, and 1915), who reported 24 new species from the Magellanic region. However, the descriptions of many of them were poor and lacking in details and 17 were unfigured; these facts resulted in several subsequent confusion and misidentifications. This is the case, for instance, of *Aligena pisum* Dall, 1908, a species described based on a single left valve. Dall's description refers to a small, suborbicular shell, "striately" sculptured, having a pale, shiny periostracum; with a prominent, slightly prosogyrate beak; an internal, opisthodetic, rather long ligament; and the hinge bearing "a single obscure tooth or nodular prominence". Although the species is mentioned in several subsequent checklists for the area (Carcelles, 1950; Carcelles & Williamson, 1951; Osorio & Bahamonde, 1970; Bernard, 1983; Ramírez Böhme, 1993), there were no additional records. Harry (1969), in the context of a systematic revision of the genus *Aligena* Lea, 1846, concluded that *Aligena pisum* was wrongly located under this genus, and proposed its tentative reallocation in the genus *Axinulus* Verrill & Bush, 1898, a decision not followed by Ramírez Böhme (1993), who retained the species under *Aligena*. Bernard (1983) considered *Aligena pisum* as a *nomen dubium*.

The genus *Adontorhina* was proposed by Berry (1947) to include Lower Pleistocene specimens of his new species *A. cyclia*. The species was subsequently reported as also living by Howard (1952). Other species currently regarded under *Adontorhina* are: *A. sphaericosa* Scott, 1986, *A. lynnae* Valentich Scott (in Coan, Valentich Scott & Bernard), 2000, *A. keegani* Barry & McCormack, 2007, and *A. similis* Barry & McCormack, 2007.

In this paper, *Aligena pisum* is redescribed, properly illustrated, and reallocated to *Adontorhina*.

MATERIALS AND METHODS

The specimens reported in this paper were collected during several field trips in southern Chile, Beagle Channel, and Magellan Strait. Samples were obtained by using a dredge of 2 mm mesh size net, and sorted from the sediment by using a stereoscopic microscope. The number of specimens (s), articulated valves (av), and disarticulated valves (v) is provided. These specimens were deposited in the collections of the Museo de La Plata (MLP) and Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Argentina. For comparative purposes, the holotype of *Aligena pisum*, housed at the United States National Museum (USNM), Smithsonian Institution, Washington, was studied.

Shell morphology was studied and illustrated with Scanning Electron Microscopy (SEM). Shell measurements were taken according to the following criteria: L: maximum antero-posterior distance; H: maximum dorso-ventral distance, perpendicular to L; W: maximum distance across closed valves. The mean and standard deviation for H/L and W/H ratios, and the number of measurements (n), are given.

Information on gross anatomy comes from dissection under stereoscopic microscope of decalcified specimens; decalcification was performed by rinsing the specimens in 5% formalin with 2% acetic acid.

SYSTEMATICS

Thyasiridae Dall, 1900

Adontorhina Berry, 1947

Type species: *Adontorhina cyclia* Berry, 1947 (OD)

Adontorhina pisum (Dall, 1908), new combination

(Figs. 1–17)

Type Locality: 53°01'00"S,70°42'15"W, *Albatross* station 2778, Magellan Strait, 61 fathoms [111.6 m], mud.

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[1]Departamento Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 4° piso, (C1428EHA) Ciudad Autónoma de Buenos Aires, Argentina
[2]División Zoología Invertebrados, Museo de La Plata, Paseo del Bosque s/n, 1900 La Plata, Buenos Aires, Argentina
*Corresponding author: mguller@bg.fcen.uba.ar

Material Examined: Magellan Strait: Holotype (USNM 110715); 53°41'S, 72°20'W, Bahía Nash, 25 m (3v, MLP 13276); 53°38'S, 72°24'W, Seno Ballena, 35 m (1s, MLP 13277). Beagle Channel: 54°53'S, 67°52'W, Isla Gable, 66–68 m (12s, MACN-In 38183); 54°52'S, 68°02'W, Punta Segunda, 168 m (7s & 1v, MACN-In 38183); 54°49'S, 68°13'W, Bahía Ushuaia, 138–144 m (25s, 1av & 5v, MLP 13278); 54°49'S, 68°14'W, Bahía Ushuaia, 126–135 m (8s & 2v, MLP 13279); 54°53'S, 68°14'W, 53–91 m (1s, MACN-In 38182); 54°52'S, 68°32'W, Bahía Lapataia, 27–29 m (1s & 1a, MACN-In 38185). Southern Chile: 42°22'S, 72°25'W, Fiordo Comau, 73–89 m (14s, 2av & 13v, MLP 13280; 10s, 7av & 11v, MACN-In 38186); 42°15'38"S, 72°25'53"W, Fiordo Cahuelmo, 70–113 m (1s, MLP 13281).

Known Distribution: Magellan Region, from Chiloé, Chile, to Tierra del Fuego. Living specimens: 27–168 m (Fig. 1).

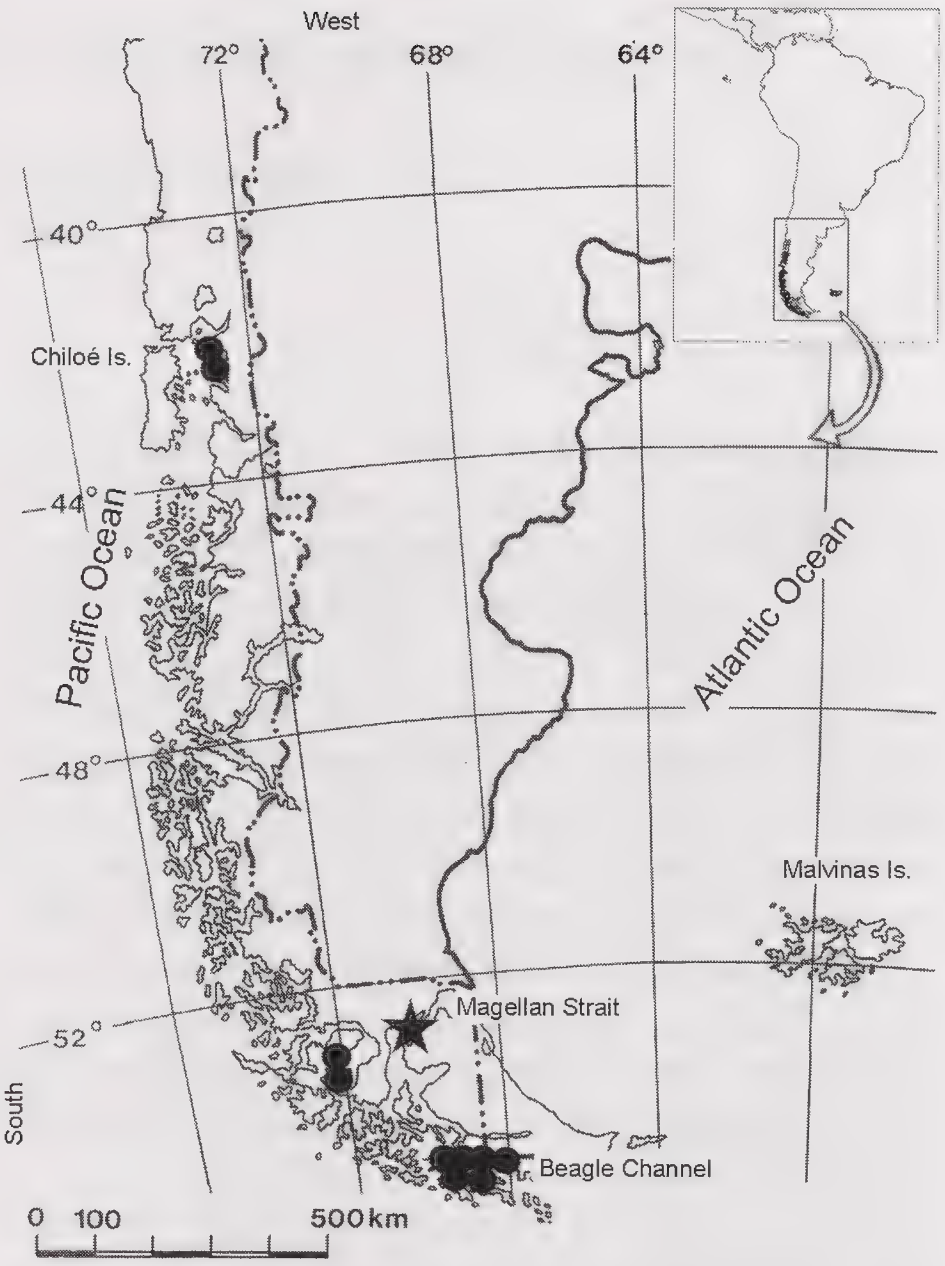
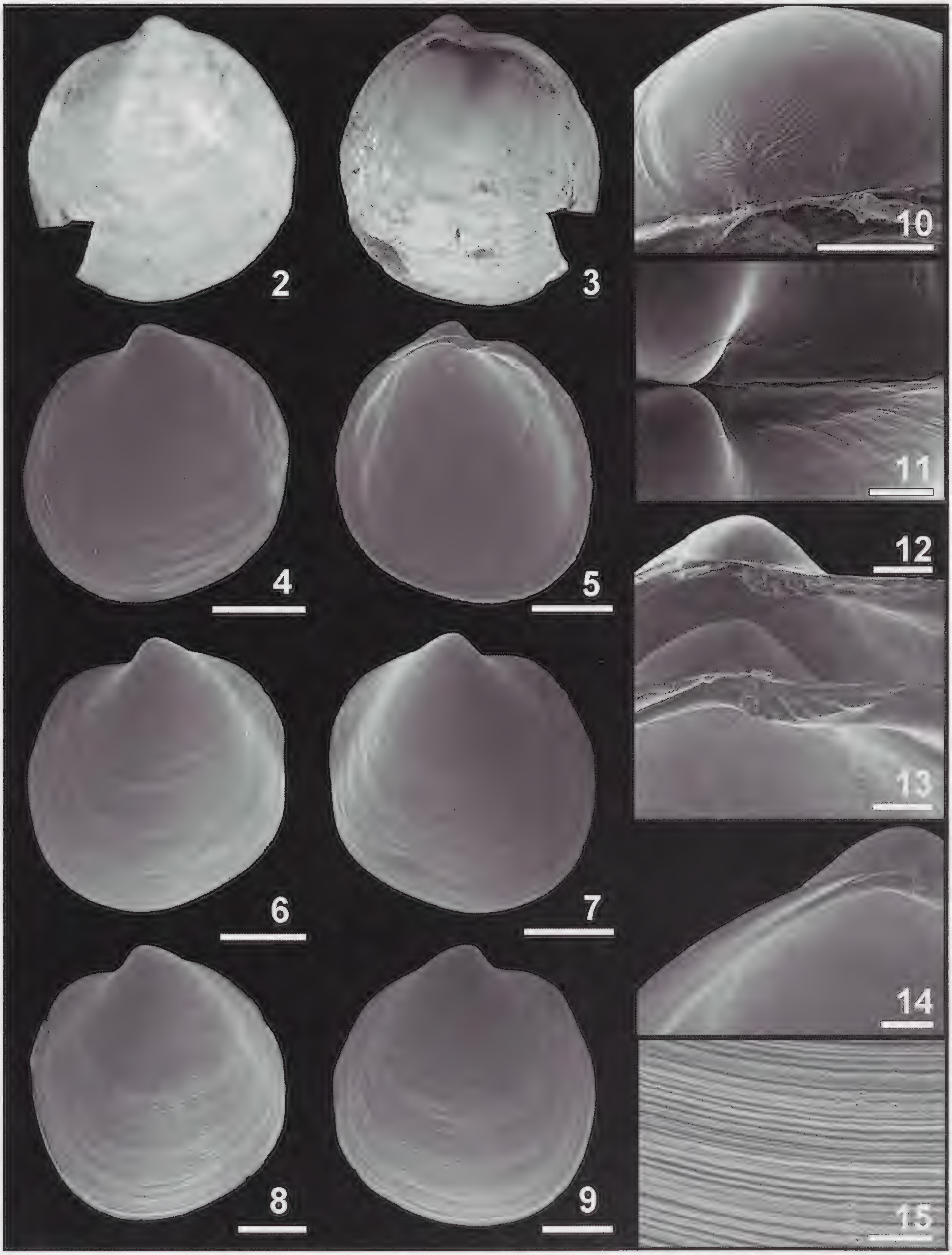


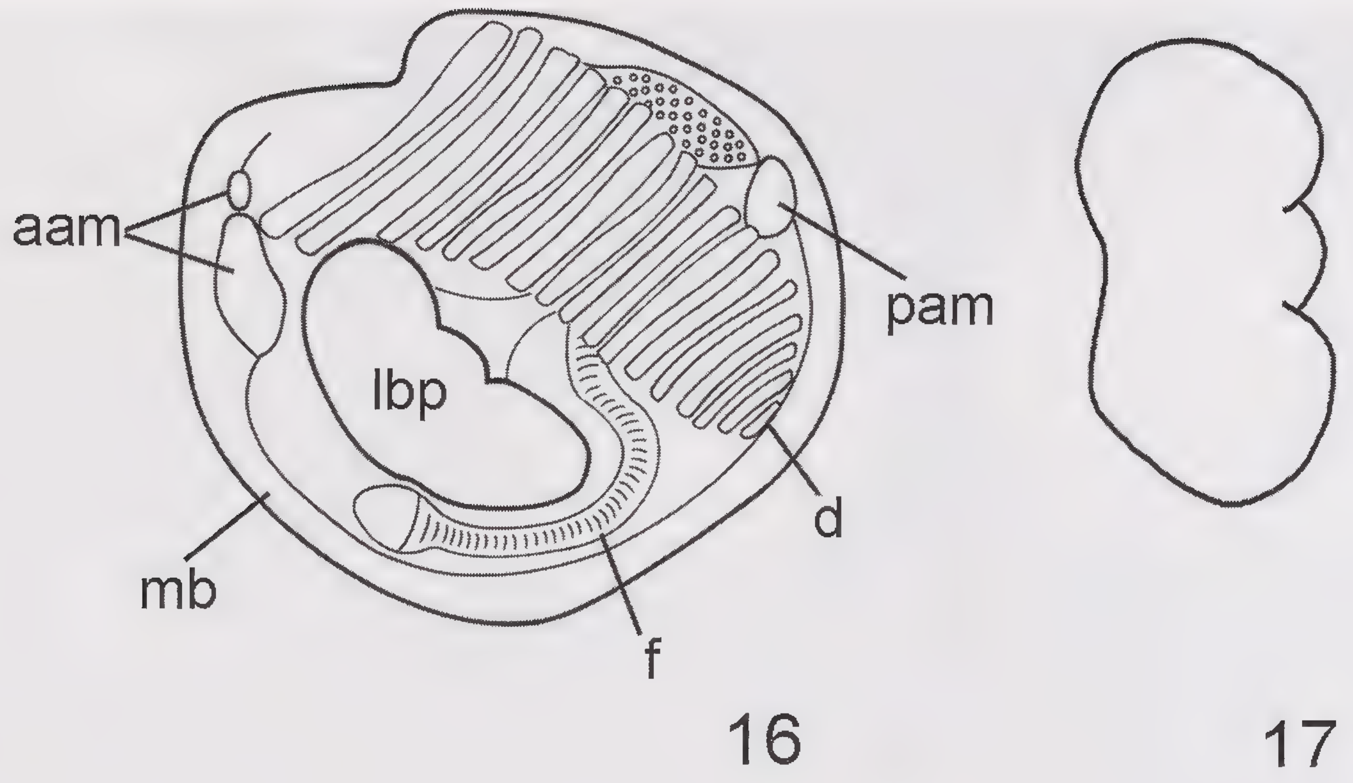
FIG. 1. Known distribution of *Adontorhina pisum*: type locality (★), other material examined (●).

Redescription: Shell small (maximum observed H = 2.5 mm), subquadrate (H/L = 1.00 ± 0.04, n = 28), somewhat inflated (W/H = 0.65 ± 0.04, n = 28), inequilateral, delicate, whitish, shiny. Anterior end higher than posterior end, moderately to widely ventrally projecting; posterior end truncate. Anterodorsal margin short, straight, nearly horizontal, forming well-marked angle at junction with anterior margin (Figs. 2–9). Dorsal part of anterior margin obliquely flattened, originated by wide depression on shell, anterior to beak; ventral part of anterior margin obliquely straight to widely arcuate, sometimes forming angle at junction with the dorsal part (Figs. 4–9). Ventral margin widely curved, markedly projected anteriorly. Posterodorsal margin short, sloping nearly straight, forming variably developed angle with posterior margin (Figs. 4–9). Posterior area well discernible from central area. First posterior fold wide, low; posterior sulcus shallow, producing straight to weakly sinuate posterior margin (Figs. 4, 6). Second posterior fold ill defined. Submarginal sulcus deep, producing variably developed submarginal sinus. Auricle moderately projecting (Figs. 4–9). Lunule narrow, elongate (Fig. 11). Beaks prominent, inflated, subcentrally located, anteriorly directed. Prodissoconch ovate, about 125 µm in diameter, delimited by low cord; initial part sculptured with numerous branched folds, almost symmetrically distributed from main central axis (Fig. 10). Shell surface sculptured with fine, low, regularly distributed comarginal cords, increasing in solidness (Fig. 15). Anterior and posterior ends frequently incrusted with ferruginous material. Periostracum thin, translucent. Hinge plate consisting of two parts: anterior with irregular, elongate granules, extending for ½ to ⅔ of dorsal margin length. Granules strong, radiating ventrally beneath beaks, and slender and parallel to dorsal margin, anteriorly, forming distinct individual patterns (Figs. 12, 13). Posterior part of hinge plate smooth (Fig. 14). Ligament, elongate, about one half the length of posterodorsal margin, deeply sunken, not visible externally.

Anatomy: Mantle margin largely free, for about ¾ of its length, widened anteroventrally (Fig. 16). Small posterior exhalant aperture, present. Transverse section of adductor muscles ovate, anterior larger than posterior. Only one (the inner) demibranch present, comprising 15–17 filaments in larger specimens (Fig. 16); ascending and descending lamellae similar in size. Lateral body pouch relatively large, with an anterior median constriction, and 3–5 posterior lobes (Fig. 17). Foot elongate, with distinct bulbous tip (Fig. 16).



FIGS. 2–15. *Adontorhina pisum*. Shell morphology. FIG. 2: Outer view, left valve, holotype (USNM 110715); FIG. 3: Inner view, left valve, holotype (USNM 110715); FIG. 4: Outer view, left valve; FIG. 5: Inner view, left valve; FIGS. 6, 8: Outer view, left valve; FIGS. 7, 9: Outer view, right valve; FIG. 10: Prodissoconch; FIG. 11: Lunule; FIG. 12: Hinge plate; FIG. 13: Anterior part; FIG. 14: Posterior part; FIG. 15: Shell sculpture. Collecting sites = FIG. 4: Magellan Strait, 53°38'S, 72°24'W, 35 m (MLP 13277); FIGS. 5, 13: Southern Chile 42°22'S, 72°25'W, 73–89 m (MLP 13280); FIGS. 6–12, 15: Beagle Channel 54°49'S, 68°14'W, 126–135 m (MLP 13279); FIG. 14: Beagle Channel 54°49'S, 68°13'W, Bahía Ushuaia, 138–144 m (MLP 13278). Scale bars: FIGS. 4–9 = 500 μm; FIGS. 10, 15 = 50 μm; FIGS. 11–14 = 100 μm.



FIGS. 16, 17. *Adontorhina pisum.* Anatomy. FIG. 16: Gross anatomy of a decalcified specimen; FIG. 17: Detail of left lateral body pouch. Abbreviations: aam = anterior adductor muscle, d = demibranch, f = foot, lbp = lateral body pouch, mb = mantle border, pam = posterior adductor muscle.

DISCUSSION

Generic Placement

The hinge of *Adontorhina pisum* was previously misunderstood. Dall (1908) described it as having "a single obscure tooth or nodular prominence", and consequently placed the species under *Aligena*. Harry (1969) regarded such prominence as "a single irregular tooth ... immediately below and in front of the umbo. The hind part of the tooth may be a broken, finger-like projection, but it appears to have several rounded bosses, one of which is on the top side. A triangular extension forward of this tooth, has a moderately sharp ventral border, along which are several incised lines sloping upward and backward". Based on this interpretation, Harry (1969) tentatively reallocated the species to *Axinulus*. However, the hinge plate in *Axinulus* is completely smooth or bears a single "cardinal" tubercle (Scott, 1986). This is not the condition present in "*Aligena*" *pisum*, where several "granules" appear. These granules are stronger beneath the beak – corresponding to what Harry (1969) interpreted as "rounded bosses" – and anteriorly become slender, running parallel to the dorsal margin (i.e., the "triangular extension" and "incised lines" of Harry (1969)). This peculiar hinge plate morphology reveals that the species actually belongs to *Adontorhina*, the single thyasirid genus having irregular granules (Scott, 1986; Barry & McCormack, 2007).

Comparison with Other *Adontorhina* Species

Adontorhina pisum closely resembles *A. cyclia*, from which it differs by having subquadrate shell outline, horizontal anterodorsal margin, and wide depressed area anterior to beak. In addition, the granules present in the anterior part of hinge plate of *A. pisum* are elongate and narrow, whereas those of *A. cyclia* are shorter and solid, resembling "mammalian molars", such as described by Scott (1986). Furthermore, the foot of *A. pisum* has a distinct bulbous tip which, according to Scott (1986), is absent in *A. cyclia*.

Adontorhina lynnae, *A. keegani*, and *A. similis* differ from *A. pisum* in their ovate shell outline, originated by their widely projected anterior end. According to Barry & McCormack (2007) the hinge plate of *Adontorhina similis* has an additional "small cardinal tubercle in the right valve", which is absent in *A. pisum*; and both *A. similis* and *A. keegani* have a minor number of gill filaments than *A. pisum* (seven to eight in *A. keegani*; 10–11 in *A. similis*; 15–17 in *A. pisum*).

Adontorhina sphaericosa differs from *A. pisum* by having a more inflated shell outline (W/H = 0.78 ± 0.04, n = 16) and deeply sunken escutcheon.

In addition to the above-mentioned differences, *Adontorhina pisum* differs from all other *Adontorhina* species by having the posterior part of hinge plate smooth. This condition enlarges the diagnostic characters previously regarded for *Adontorhina*, which consequently encompasses species with either a smooth or granulated posterior part of the hinge plate.

Significance of the Record

The reallocation of *Aligena pisum* into *Adontorhina* significantly increases the geographic distribution of *Adontorhina*. The genus was previously reported from the Northern Hemisphere in the North Atlantic Ocean [*Adontorhina keegani* and *A. similis* from Porcupine Bank, west of Ireland (Barry & McCormack, 2007)], and in the North Pacific Ocean [*A. cyclia*, from the Bearing Sea, Alaska to Baja California, Mexico (Scott, 1986), and from Bering Sea, Sea of Okhotsk, and Sea of Japan (Kamanev, 1996); *A. sphaericosa* from Alaska to California (Scott, 1986; Coan et al., 2000); and *A. lynnae* from near the Farallon Islands, California (Coan et al., 2000)]; and an as-yet undescribed species in the Gulf of California, Mexico (Coan, personal communication, 7 Oct. 2010). Thus, *Adontorhina pisum* provides the first record of the genus in the Southern Hemisphere. In addition, this finding increases the diversity of thyasirids known from the Magellan Region, previously represented only by *Thyasira* Lamarck, 1818; *Parathyasira* Iredale, 1930; *Conchocele* Gabb, 1866; and *Mendicula* Hedley, 1907 (Zelaya, 2009, 2010).

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HISTORICAL NOTE

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HOW THE NAIAD WAS DRAWN: A PRE-LINNEAN ICONOGRAPHY OF FRESHWATER MUSSELS

Arturo Valledor de Lozoya[1] & Rafael Araujo[2]*

ABSTRACT

This paper compiles the pre-Linnean iconography of freshwater mussels or naiads and provides an understanding of the historical framework in which they were created. The first book providing a figure of a freshwater mussel would seem to be *Hortus sanitatis*, an anonymous encyclopaedia first published in 1491. This and other early images of these animals related to the activity of obtaining pearls from *Margaritifera margaritifera*. The first traditional image of a freshwater mussel, drawn by Rondelet in 1555, was thereafter used by such other early naturalists as Boussuet, Gessner, Aldrovandi, and Jonston. The first microscopists Heide and Leeuwenhoek and pioneer malacologists Lister, D'Argenville and Ginanni were the first to depict the anatomy of naiads. We also have discovered a pre-Linnean image of a living naiad by Poupart showing its typical furrow in the sediments, and a pencil sketch by Linnaeus showing a pearl fishery of *M. margaritifera*.

Key words: pre-Linnean, history of malacology, Margaritiferidae, Unionidae, naiads.

INTRODUCTION

Long before a freshwater mussel or naiad was ever illustrated, the fossil record reflects a relationship between man and these bivalves. A recent analysis of aquatic fossils associated with hominids in the early Pleistocene of Trinil and Sangiran, Java, where the first fossil bones of *Homo erectus* were found, suggests that specimens of several freshwater mussel species from the nearby Solo River could have been deliberately caught by this hominid for consumption (Joordens et al., 2009), supported by the presence of many paired valves indicating that the molluscs were not dead and were transported by water before fossilization and by the complete absence of juvenile specimens, suggesting size-selective collection.

Later middens or accumulations of freshwater mussel shells have been discovered in ancient settlements of five continents, again suggesting that the soft parts of these animals were eaten. The shells could also be used as vessels, spoons, or other tools. Those middens include archaeological sites of the Palaeolithic, Neolithic, and Bronze ages in many parts of Europe (Locard, 1885; Hertel, 1956; Harrison et al., 1994; Cochet, 2005; Borrello & Girod, 2008) and Turkey, Syria and Jerusalem (Reese, 1990, 1995; Cartwright, 2003). They appear as well in Roman settlements (Sandberger, 1873; Kuipjer, 1988), Moorish settlements in Spain (Lozano et al., 2004), Egyptian tombs (Burleigh, 1983), Indian settlements in North America close to the Mississippi River and its tributaries (Matteson, 1953a, b; Hughes & Parmalee, 1999; Warren, 2000; Davis & Muehlenbachs, 2001), and Australian aboriginal settlements (Richards et al., 2007). Even today, freshwater mussels are a traditional source of food for Australian aborigines.

The first known shell collection, found at Pompeii and now housed in the Archaeological Museum of Naples, Italy, includes the freshwater mussel *Anodonta cygnea* (Linnaeus, 1758) (Damon, 1867; Tiberi, 1879; Dance, 1986).

Besides their use as food, the capacity of freshwater mussels to produce pearls has also generated great interest. Pearls were generically known in the Roman Empire as *margaritae*, but the word used for those of an exceptional size or quality was *unio*, which were unique and very valuable objects. The Romans imported marine pearls from the Red Sea and the Persian Gulf, but at least four Roman authors briefly mention the freshwater pearls of Britain: Gayus Suetonius (transl., 2004), Cornelius Tacitus (transl., 2010), Clau-

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[1]Costa Brava 33, 28034 Madrid, Spain
[2]Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain
*Corresponding author: rafael@mncn.csic.es

dius Elianus (transl., 1984), and Gayus Plinius Secundus, better known as Pliny the Elder (transl., 1624; ed. 1992). Suetonius pointed out that, given his enthusiasm for pearls, Julius Caesar's main reason for invading Britannia in 55 B.C. was to gain control of its pearl rivers. According to Pliny, Caesar went to the temple of *Venus Genetrix* and dedicated to this Roman goddess a rich breastplate decorated with British pearls.

It was not, however, until the 13th century that the use of the word *pearl* and its French and Spanish variants *perle* and *perla*, all derived from the Latin *pirula* meaning "small pear", became the general term throughout Europe, replacing the word *margarita*. During the late Middle Age, European jewellers distinguished between so-called Oriental pearls, produced by the marine species *Pinctada margaritifera* (Linnaeus, 1758) and *P. radiata* (Leach, 1814), from the freshwater pearls produced by the European freshwater mussel *Margaritifera margaritifera* (Linnaeus, 1758). According to a document dated 1355, it seems that freshwater pearls were then preferred over marine pearls from India, the Red Sea, and the Persian Gulf, since the jewellers of Paris resolved not to mix the two types of pearl in items made of gold and silver, although an exception was made for large works for religious use (Landman et al., 2001).

The fishing and trading of the freshwater pearls of *M. margaritifera* since at least the 16th century are documented in Scandinavian rivers (Magnus, 1555), Scottish rivers (Geiger, 1637), and the Elster River and its tributaries in the Saxony Highlands (Rudau, 1961). All the pearls then fished in these and other European countries with *M. margaritifera* fisheries, such as Bavaria, Silesia, Bohemia, and Russia, were the property of emperors and kings, and there were heavy punishments for anyone found poaching.

In China, the earliest reference to freshwater pearls dates back to more than 4,000 years, according to the book on historical facts *Shu King*, in which King Yu is described to have received pearls from the Hwai River as a tribute (Donkin, 1998). The Chinese obtained marine pearls from southern China or imported them from Southeast Asia and the Persian Gulf, but even in remote antiquity, probably as early as the second or third century B.C., they had access to pearls from the freshwater mussels *Cristaria plicata* (Leach, 1814) and *Hyriopsis cumingii* (Lea, 1852), which are native to the Yangtse Delta and the rivers of central China. The species *M. margaritifera* was also exploited in the rivers of Manchuria and Korea, where there are records of the pearl extraction since the 14th century (Ziuganov et al., 1994).

There do not seem to be any representations of freshwater mussels by the old masters despite the use of the shells of these mussels, with their impermeable nacre lining, as vessels for mixing pigments and keeping the expensive golden powder. This use led to the designation *Conchae pictorum* [shells of painters] for naiads by almost all pre-Linnean naturalists. Later, Linnaeus chose the word *pictorum* to denote the *Unio pictorum* and assigned the name *anatina* [of ducks] to another naiad species in the genus *Anodonta*, alluding to its common use in some regions of Europe to feed ducks and pigs. In addition, he gave the name *cygnea* [of swans] to a similar, more slender and larger species of *Anodonta*.

Although a relationship between freshwater mussels and man is evident throughout prehistory and history, the first illustration of a specimen of this group of molluscs did not appear until the end of the 15th century. In the present paper, we show this and other figures to contribute to our knowledge of these animals and of pre-Linnean natural history.

MATERIAL AND METHODS

A search was conducted for images of naiads in friezes, sculptures, mosaics, bas-reliefs, and mural pictures from prehistory and Assyrian, Egyptian, Persian, Indian, Chinese, Greek, Etruscan, and Roman cultures. We also examined Gothic, Renaissance, and Baroque paintings, paying special attention to those of painters who commonly represented animals, such as Albrecht Dürer, Joris Hoefnagel, Daniel Fröschl, Rolandt Savery, Jan van Kessel, and other Flemish artists. Many drawings and engravings from the 16th to the 18th centuries were also checked for images of naiads, including old iconographical collections, such as the Van Berkheij's at the Museo Nacional de Ciencias Naturales, Madrid, Spain.

In an exhaustive bibliographic search, we reviewed books and documents prior to the printing in 1758 of the 10th edition of *Systema naturae* by Linnaeus, officially considered the start of zoological nomenclature. Due to the vast amount of literature examined, most of the old books and papers examined are not cited.

TABLE 1. Information on the pre-Linnean iconography of freshwater mussels.

Figure	Author	Date	Species name in the original	Modern name of species
Fig. 1	Anonymous	1491	*Eceo la concha margaritifera*	*Margaritifera margaritifera*
Fig. 2	Anonymous	1491	Untitled	*Margaritifera margaritifera* (pearl fishing)
Fig. 3	Magnus	1555	*De Margaritis & earum generatione*	*Margaritifera margaritifera* (pearl fishing)
Fig. 4	Geiger	1637	*Scottorum in fluviis piscandi modus*	*Margaritifera margaritifera* (pearl fishing)
Fig. 5	Rondelet	1555	*Musculis aquae dulcis*	*Anodonta* sp. (*anatina*?)
Fig. 6	Boussuet	1558	*Musculis aquae dulcis*	*Anodonta* sp. (*anatina*?)
Fig. 7	Gessner	1558	*Musculis aquae dulcis Rondeletius*	*Anodonta* sp. (*anatina*?)
Fig. 8	Aldrovandi	1606	*Musculus alter*	*Anodonta* sp. (*anatina*?)
Fig. 9	Jonston	1650	*Musculus Muschel*	*Anodonta* sp. (*anatina*?)
Fig. 10	Gessner	1558	*Conchae longae species, etc.*	*Margaritifera margaritifera*
Fig. 11	Aldrovandi	1606	*Concha longa zoographi*	*Margaritifera margaritifera*
Fig. 12	Lister	1678	*Musculus latus & Musculus angustior*	*Anodonta anatina & Unio pictorum*
Fig. 13	Buonanni	1681	*Concha longa, pictorum dicta*	*Unio pictorum*
Fig. 14	Buonanni	1709	*Concha longa, pictorum dicta*	*Unio pictorum*
Fig. 15	Lister	1681	*Musculus, etc.*	*M. margaritifera, A. anatina. & U. pictorum*
Fig. 16	Lister	1685–1692	*Musculus niger, etc.*	*Margaritifera margaritifera*
Fig. 17	Lister	1685–1692	*Musculus angustior, etc.*	*Unio pictorum*
Fig. 18	Lister	1685–1692	*Musculus latus, maximus, etc.*	*Anodonta* sp. (*cygnea*?)
Fig. 19	Lister	1685–1692	*Musculus tenuis, minor, etc.*	*Anodonta anatina*
Fig. 20	Lister	1685–1692	*Musculus fluviatiles, é Fluvio Tamesi*	Deformed specimen
Figs. 21–26	Lister	1685–1692	*Musculus, etc. & Pectunculus, etc.*	Several unidentified species
Fig. 27	Lister	1685–1692	*Pectunculus subviridis, triquetrus*	See comment on this figure in text
Fig. 28	Lister	1685–1692	*Musculus, a Danubio*	*Unio tumidus*
Fig. 29	Lister	1696	*Musculi fluviatilis anatome*	Unionidae (anatomy, glochidia, shell crystals)

(continues)

(continued)

Figure	Author	Date	Species name in the original	Modern name of species
Not figured	De Heide	1683	*Mytuli, Belgicè mossel*	*Anodonta anatina* (isolated parts)
Fig. 30	Van Leeuwenhoek	1697	*Veen-Oesters or Mosselen*	*Anodonta cygnea* (anatomy and embryos)
Fig. 31A	Poupart	1731	*Moule*	*Anodonta anatina*
Fig. 31B	Poupart	1731	*Moule*	Unidentified Unionidae (specimen track)
Not figured	Scheuchzer	1735	*Fluss-Muschel, Musculus Fluviatilen*	Unidentified Unionidae (specimen track)
Fig. 32	Anomymous	1736	*Hüte Dich fur Schaden*	*Margaritifera margaritifera* (pearl fishing)
Fig. 33	Anonymous	circa 1760	*Perlen-Sucher, etc.*	*Margaritifera margaritifera* (pearl fishing)
Fig. 34	Linnaeus	1732	Untitled	*Margaritifera margaritifera* (pearl fishing)
Fig. 35	Hermann	1737	*Concha fluviatiles margaritifera, etc.*	*Margaritifera margaritifera* (anatomy)
Fig. 36	Gualtieri	1742	*Musculus fluviatilis, etc.*	*Unio* sp. & *Anodonta cygnea*
Fig. 37	D'Argenville	1742	*Moules*	Several unidentified species
Fig. 38	D'Argenville	1757	*Moules*	Several unidentified species (living specimens)
Fig. 39	D'Argenville	1780	*Moules*	Several unidentified species
Fig. 40	Marsigli	1744	*Moule*	*Unio* sp.
Fig. 41	Swammerdam	1737	*Een Holandische Mossel*	Unidentified Unionidae (soft parts)
Fig. 42	Klein	1753	*Diconcha sulcata*	*Margaritifera margaritifera*
Fig. 43	Klein	1753	*Musculus latus*	*Anodonta anatina*
Fig. 44	Valentijn	1754	Untitled	*Unio pictorum*
Fig. 45	Pontoppidan	1755	*Freshwater muscle & pearls*	*Margaritifera margaritifera* (pearls)
Fig. 46	Ginanni	1757	*Conca de Pittori*	*Anodonta anatina* (soft parts)
Fig. 47	Ginanni	1757	*Conca de Pittori*	*Unio* sp. (*pictorum*?)
Fig. 48	Adanson	1757	*Le Mutel*	Unidentified Mutelidae
Fig. 49	Knorr	1768	*La Moule a perles de l'Elster*	*Margaritifera margaritifera*

The following are the types of bibliographic sources included: (1) old editions by authors who wrote about natural history, such as Aristotle (transl., 1970), Pliny, Elianus, Dioscoride, Albertus Magnus, Vincent de Beauvois, Konrad von Megenberg, Clusius; (2) Medieval and Renaissance bestiaries and *Physiologus* of Oxford, Cambridge, Westminster, Bern, El Escorial, Juan de Austria, Rudolph II of Habsburg; (3) *Cosmographies* by Münster and Thevet; (4) catalogues of the old *Kunstkammern* by Imperato, Ceruti, Tradescant, Worm, Moscardo, Terzago, Olearius, Legati, Grew, Dumolinet, Kircher, Buonanni, Valentini, Besler, Vincent, Seba, Gersaint; (5) books devoted to invertebrates or aquatic animals by Belon, Rondelet, Boussuet, Salviani, Mattioli, Gessner, Aldrovandi, Colonna, Jonston, Petiver, Redi; (6) books on gemology and margaritology by Boodt and Geiger, as well as modern books on pearls by Donkin (1998), Joyce & Addison (1993), Landman et al. (2001); (7) dissertations and opuscules on the nature of pearls and their formation by Hermann, Zschau, Oertel, Plot, Réaumur and other 18th century authors, including the information at the *Encyclopédie* by Diderot, D´Alembert, and Jaucourt; (8) other opuscules on mussels and bivalves by Heide, Poupart, Méry; (9) pre-Linnean books on malacology and conchology by Buonanni, Lister, Rumpf, Gualtieri, D'Argenville, Adanson, Knorr, Regenfuss; (10) modern books dealing with paintings of still lives and of shells.

Especially useful for our research were the bibliographic references given by Lister (1692–1697) in the second edition of *Historia conchyliorum*, by Regenfuss (1758), and by Schröter (1779). Also of great help have been the compilations of zoological iconography by Barbero (1999) and on the ancient malacological bibliography by Caprotti (1994), the catalogue of special funds of the Library of the Museo Nacional de Ciencias Naturales of Madrid (Vicente & Orbiso, 1990; Vicente et al., 1992, 1998; Orbiso et al., 2000), and electronic versions of the catalogues of the British Library, London, England, the Gallica National Library, Paris, France, the National Library of Madrid and the Library of the Consejo Superior de Investigaciones Científicas in Spain.

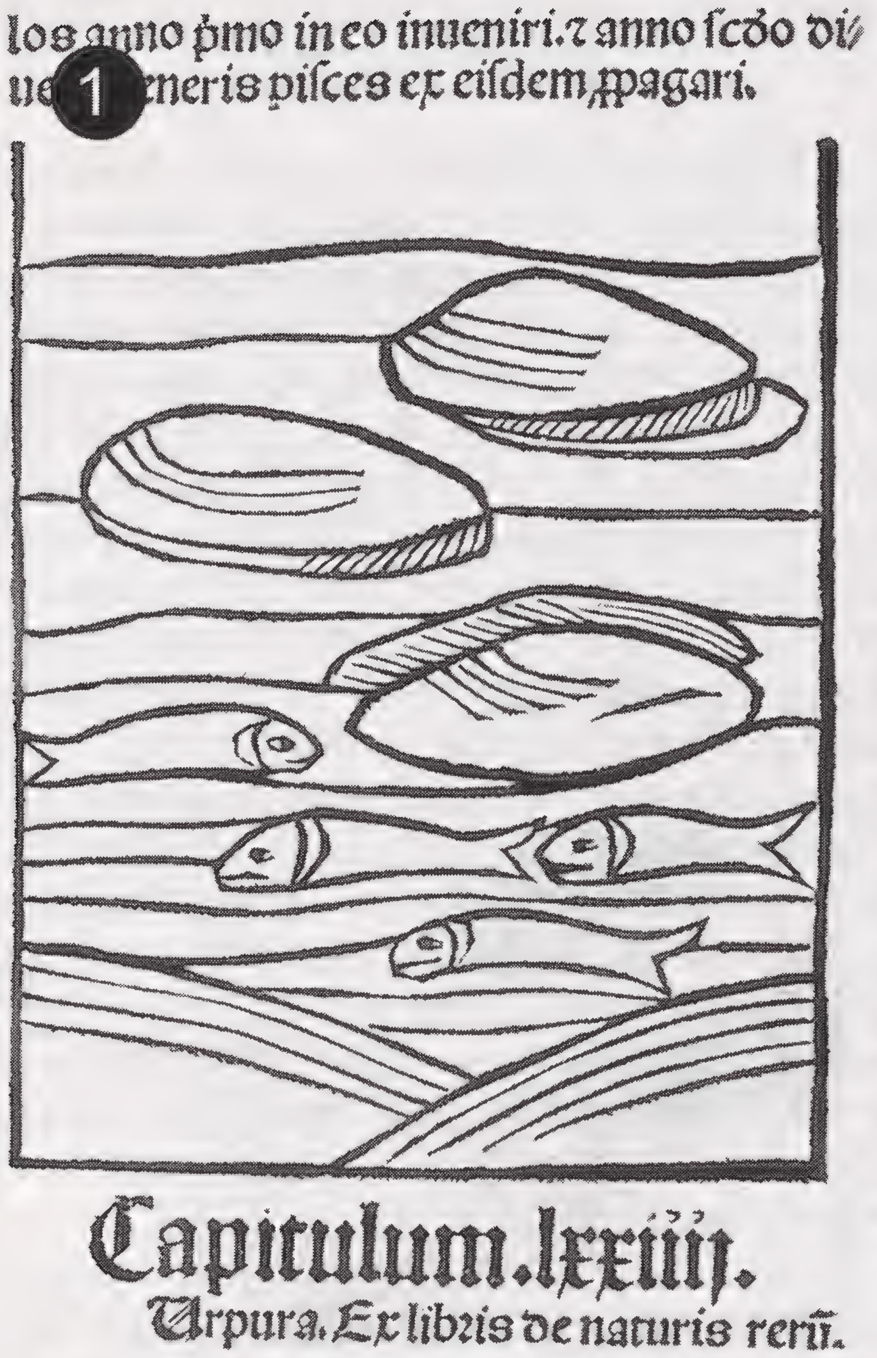
Some of the original figures reproduced here have been slightly modified for reasons of space.

PRELINNEAN ICONOGRAPHY OF FRESHWATER MUSSELS

The first book in which a figure of a freshwater mussel was provided (Table 1) seems to be *Hortus sanitatis*, an zoological and botanical encyclopaedia, first published in 1491 in Mainz, Germany, with later editions (Anonymous, 1491). This book has been claimed to have been written by a German physician named Johann Wonnecke von Caub or Johannes de Cuba. In a chapter entitled *Tractatus de piscibus*, there is a woodcut showing several fishes together with three elongate bivalves with extended mantles and feet (Fig. 1). The figure suggests today's well-known relationship between naiads and fishes. An accompanying text reads *Eceo la concha margaritifera* [here is the pearl-producing shell] alluding to *M. margaritifera*. Another picture (Fig. 2) in a chapter entitled *De lapidibus*, shows a man extracting a pearl from a mussel with another pearl in the other hand probably being subjected to the so called "tooth test".

In chronological order, the next book to consider is *Historia de gentibus septentrionalibus* by Olaus Magnus, a catholic bishop of Uppsala, Sweden, who later moved to Rome. The first edition appeared in 1555 and was followed by several others. One of its many small woodcuts showed a scene of a pearl fishery in a river under the title *De margaritis et earum generatione* [on pearls and their origin] (Fig. 3). It also showed sumptuary objects related to pearls, such as rings, strings, crowns, and jewel cases. The sun shining over the scene probably alludes to Pliny's theory on the origin of pearls, according to which mothers of pearls come to the water surface, take in the dewdrops, and generate pearls after being fertilized by a sun ray. A flying bird, probably a kingfisher, carries a naiad in its beak in another reference to this theory. Similar yet different scenes appear in other editions.

Another scene of a pearl fishery in a river can be found in *Margaritologia, sive dissertatio de margaritis*. This book, printed in 1637, was the first exclusively devoted to pearls and their medical uses. Its author was Malachias Geiger, physician to Emperor Rudolph II of Habsburg. Anselmus Boetius de Boodt (1609) was also physician to that emperor and also published a treatise on gems and pearls, but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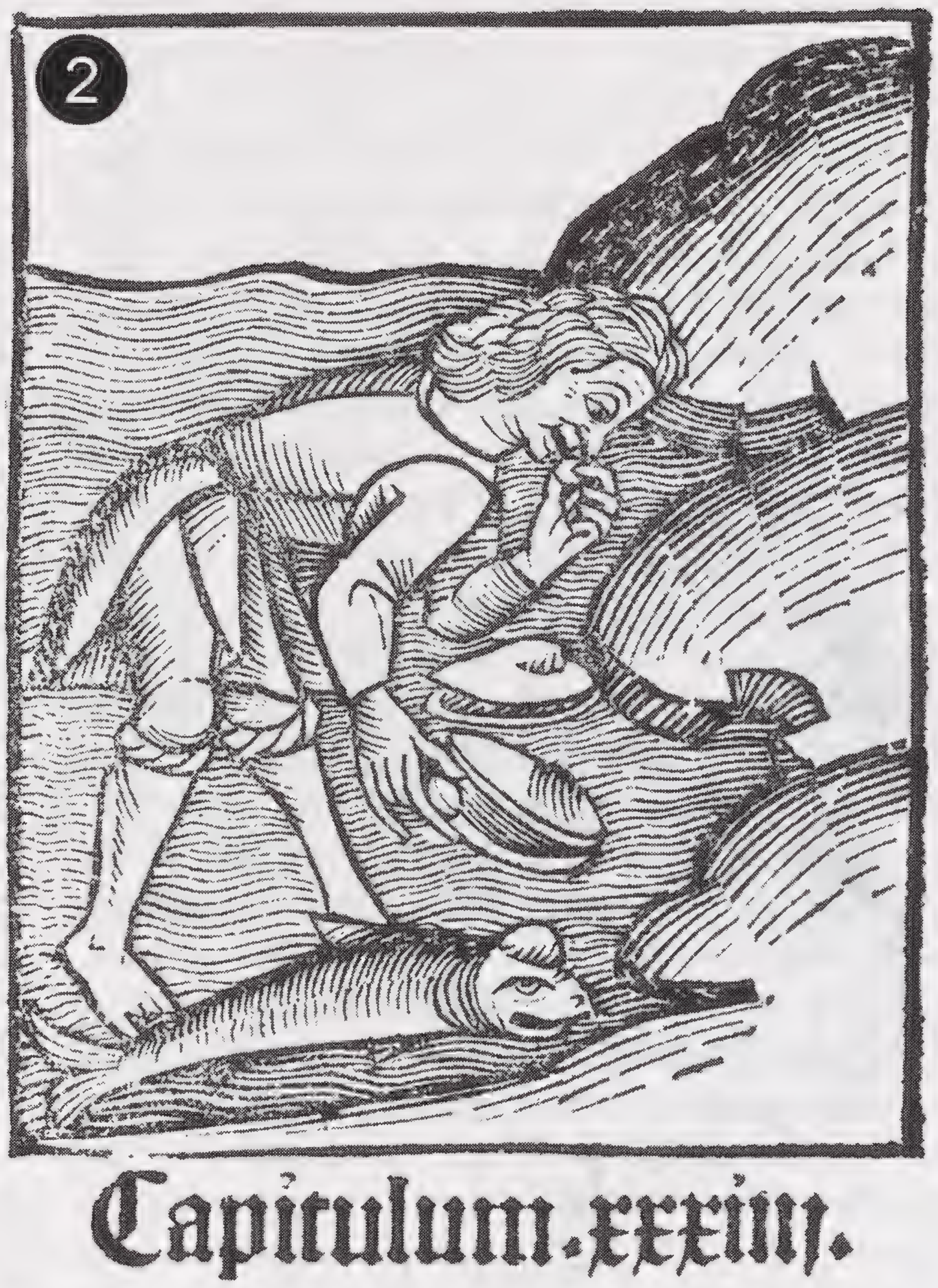
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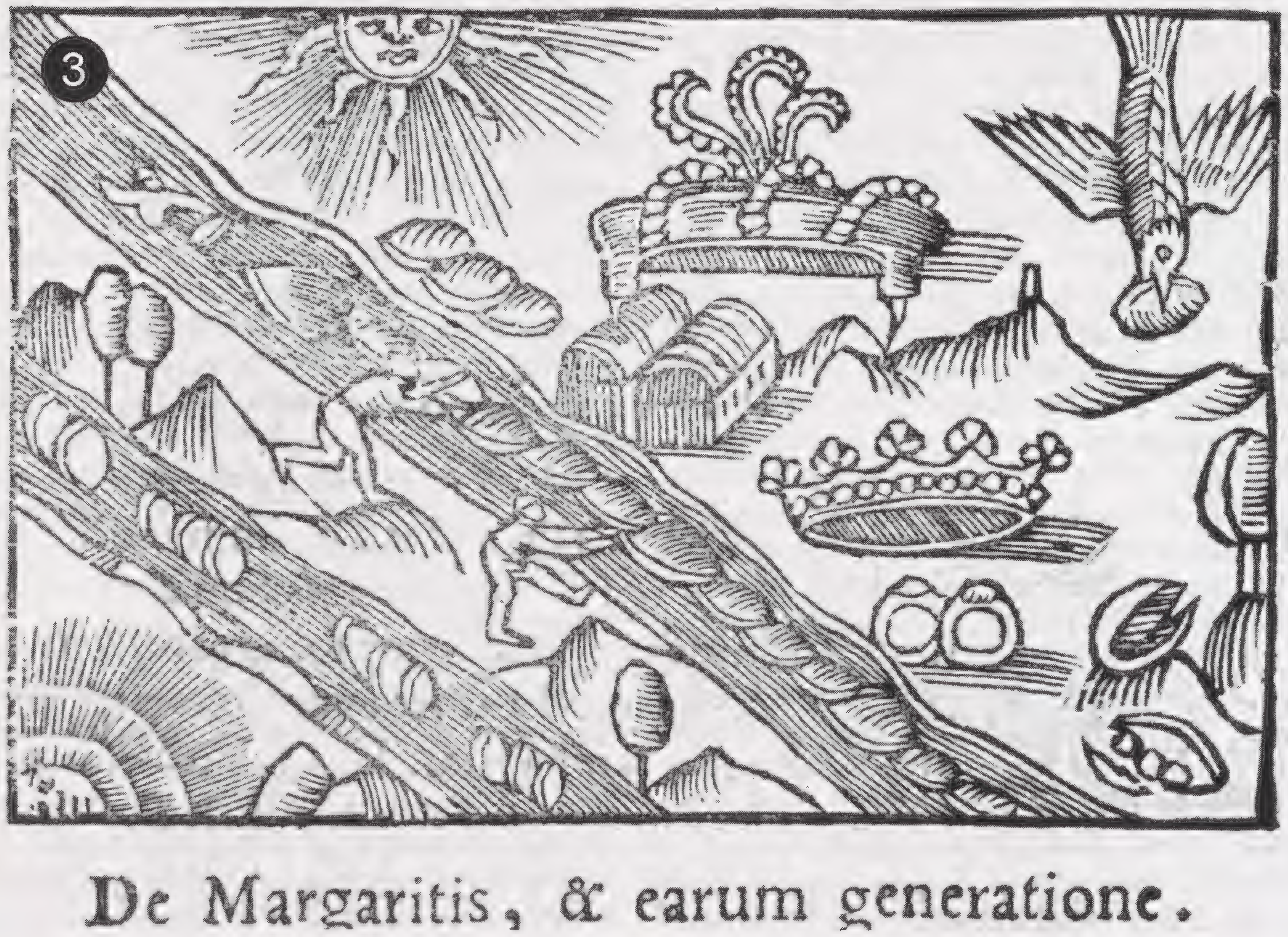
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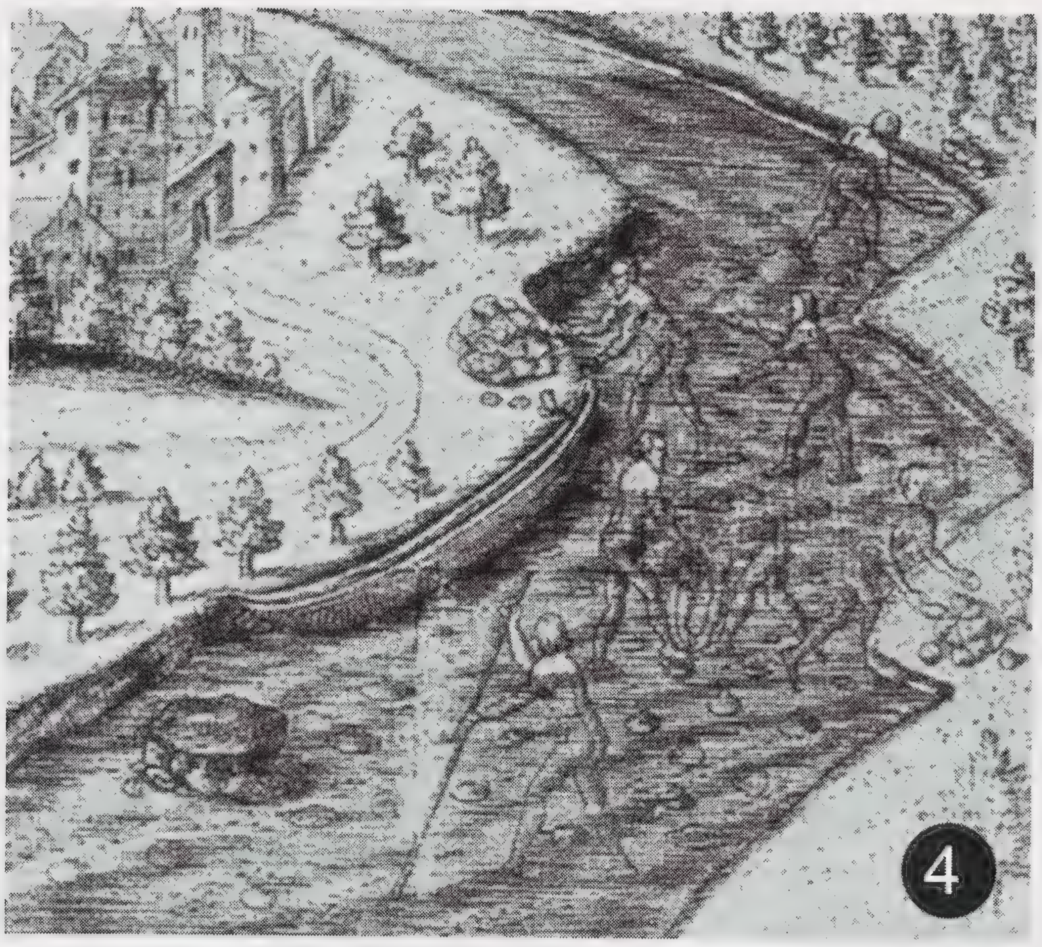
De Margaritis, & earum generatione.

FIGS. 1–3. FIG. 1: *Concha margaritifera* from *Hortus sanitatis* (Anonymous, 1491); FIG. 2: Other image supposedly representing *Margaritifera margaritifera* from *Hortus sanitatis* (1491); FIG. 3: A fishery of *Margaritifera margaritifera* from Magnus (1555). FIGS. 1–3: By permission of Biblioteca Nacional, Madrid, Spain.



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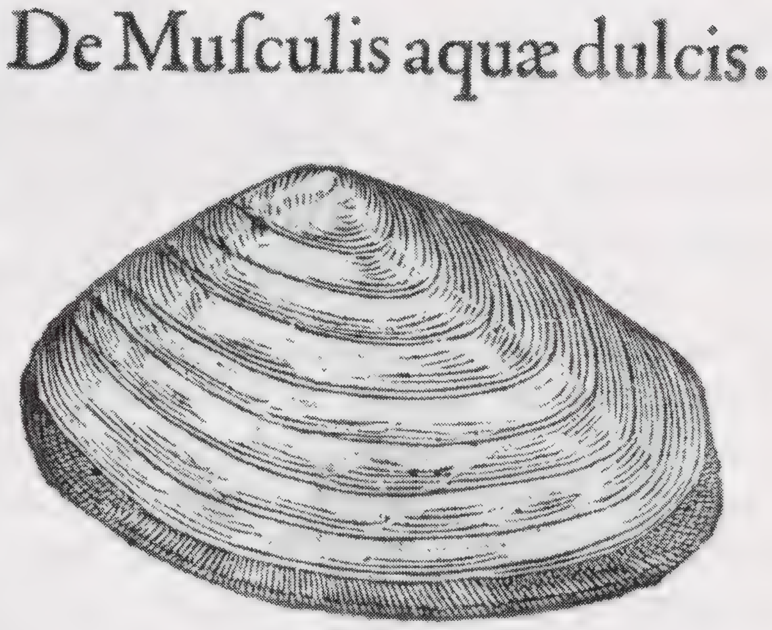
De Muſculis aquæ dulcis.

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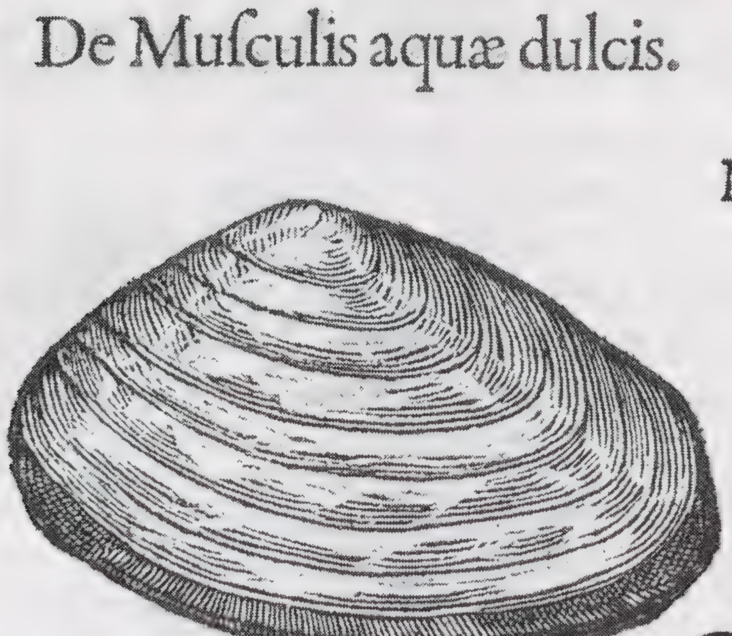
De Muſculis aquæ dulcis.

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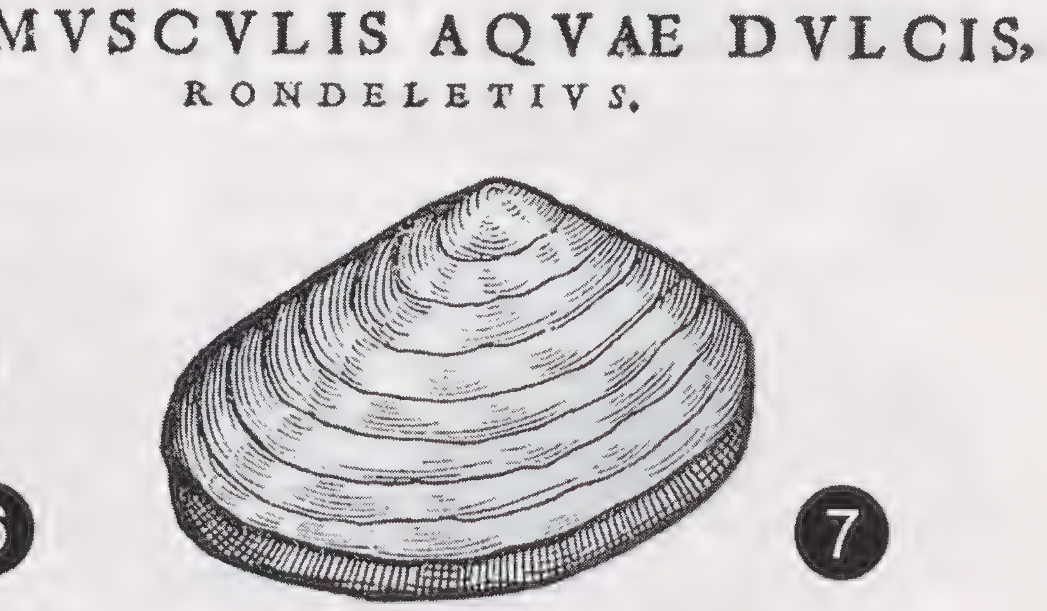

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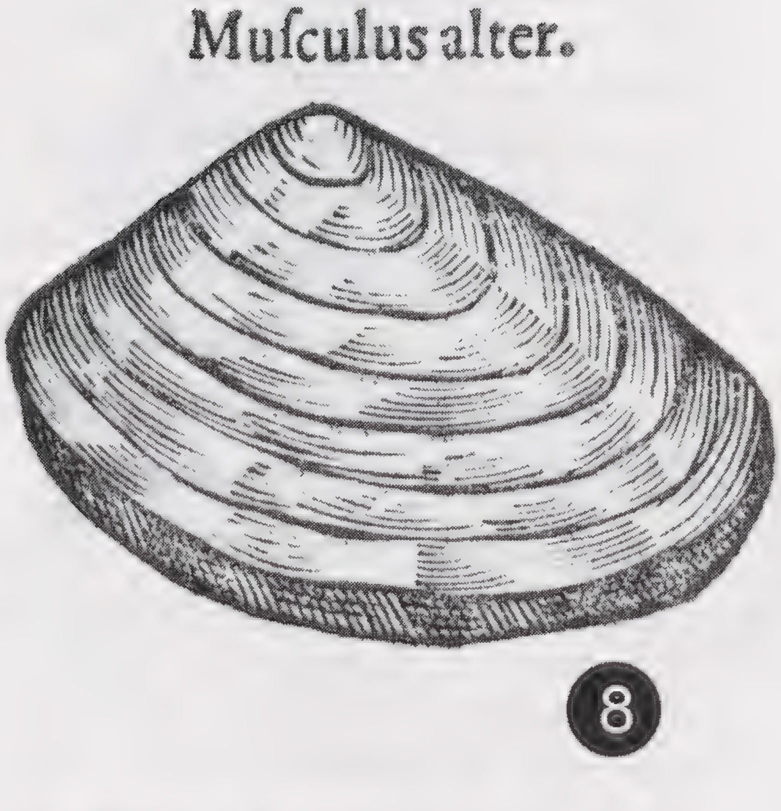
Muſculus alter.

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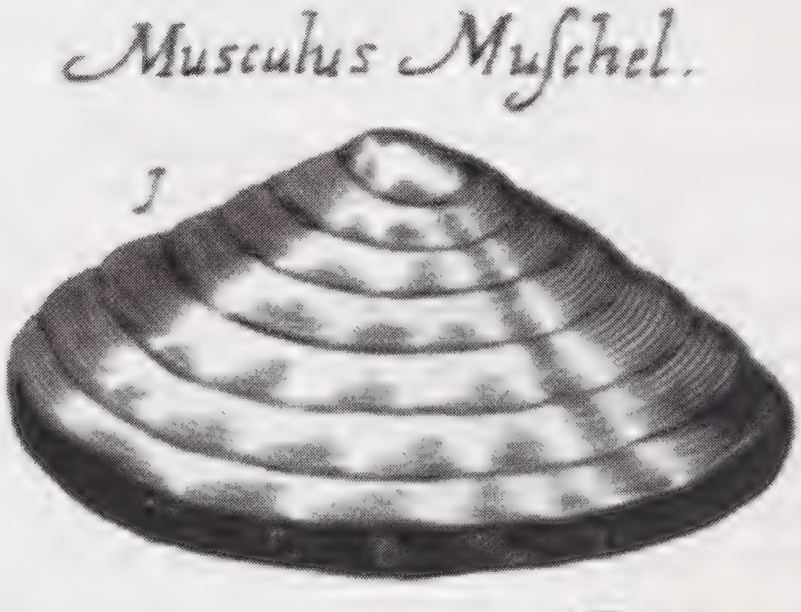

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FIGS. 4–9. FIG. 4: A fishing pearls scene in a river of Scotland from Geiger (1637); FIG. 5: *Musculus aquae dulcis* from Rondelet (1555); FIG. 6: *Musculus aquae dulcis* from Boussuet (1558); FIG. 7: *Musculus aquae dulcis* from Gessner (1558); FIG. 8: *Musculus* from Aldrovandi (1606); FIG. 9: *Musculus* or *Muschel* from Jonston (1650). FIGS. 5–8: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 9: By permission of Biblioteca Nacional, Madrid, Spain.

it lacked images of naiads. A folded plate in Geiger (1637) represents four ways of obtaining pearls in different parts of the World. The fourth manner (Fig. 4) was referred to as *Scottorum in fluviis piscandi modus* [fishing in rivers in the Scottish way].

The first canonical image of a freshwater mussel can be found in *Universae aquatilum historia* by Rondelet (1555), the second part of *Libri de piscibus marinis* by this author, who was a professor of medicine at the University of Montpellier, France. The triangular shape of a shell there named *Musculis aquae dulcis* [freshwater mussel] (Fig. 5) suggests it is a specimen of *Anodonta anatina*. The Latin text explains that its flesh is hard, difficult to cook, foul tasting and that its juices could produce fever. These last words are the first medical reference to the capacity of freshwater mussels, as filter feeding organisms, to accumulate bacteria and viruses, causing such infectious diseases as gastroenteritis, typhoid fever, cholera, and hepatitis.

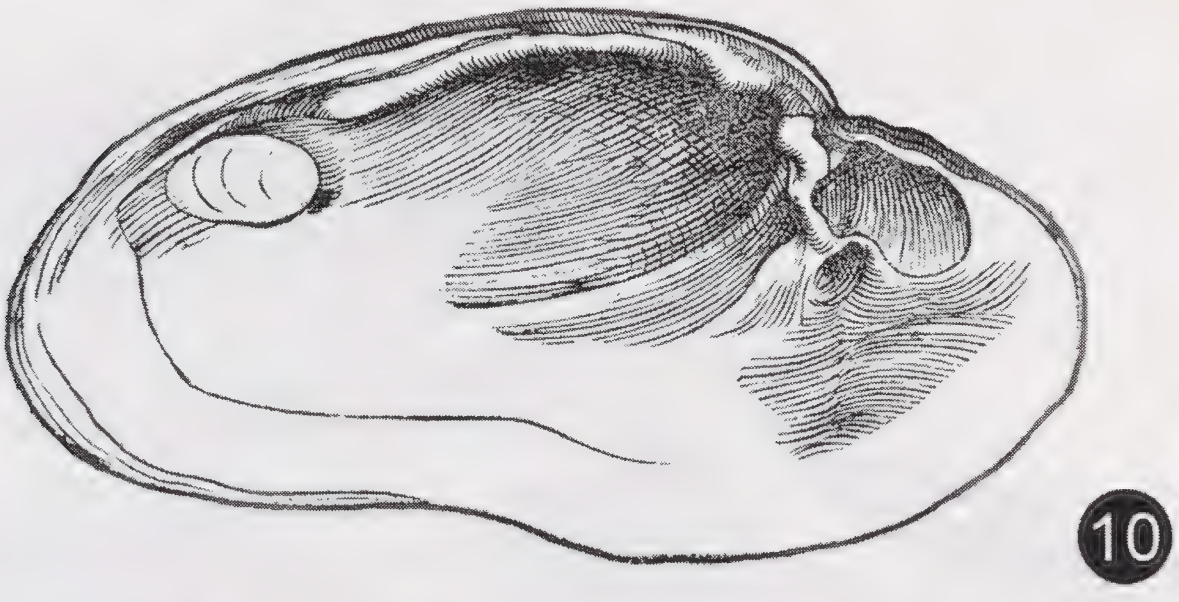
This image by Rondelet (1555) was repeated by Boussuet (1558) (Fig. 6), because Matthias Bonhomme, an editor from Lyons, France, who printed both books, used the same woodcuts to illustrate them. The picture was also copied and printed as a mirror image by the Swiss doctor and naturalist Konrad Gessner (1558) for *De aquatilibus* (Fig. 7), the fourth book of his famous *Historia animalium*, a work that saw later editions (Gessner, 1560, 1563), the first one only including the iconographical part and short commenting texts. Two more drawings of Rondelet's *Musculis aquae dulcis* were provided by Ulisse Aldrovandi (1606) (Fig. 8), professor of medicine at the University of Bologna, Italy, and by John Jonston (1650) (Fig. 9), a Polish physician and naturalist established in England. Interestingly, two other figures by Jonston named *Concha margaritifera* and *Concha pictorum* are not naiads, despite their names, but rather the marine bivalves *Pinctada margartitifera* and *Anomia ephippium* (Linnaeus, 1758).

An unmistakable, often copied image of a freshwater mussel was originally figured by Gessner (1558). This figure showed the inner side of a left valve of *M. margaritifera* (Fig. 10) with a caption reading *Conchae longae species etiam in dulcibus aquis reperitur* [long-shelled species also found in freshwater]. The attached text speaks of *Concha pictorum* [shell of painters]. As happened with the above-mentioned figure of *Musculis aquae dulcis Rondeletius* [freshwater mussel according to Rondelet], this figure by Gessner was copied by Aldrovandi (1606) (Fig. 11) and inspired later drawings by such authors as Lister, Klein, and Knorr, as discussed below.

The British physician and naturalist Martin Lister was a fellow and then vice-president of the Royal Society of London and author of a monumental malacological work. His *Historia animalium Angliae* (1678) illustrated in four figures the inner and outer sides of *Anodonta* sp. and *Unio pictorum* (Fig. 12), which Lister respectively named *Musculus latus* [broad mussel] and *Musculus angustior* [narrow mussel]. These pictures of *Unio* by Lister (1678) were used by Filippo Buonanni (1681) for his *Ricreatione dell´occhio e della mente nell´osservatione delle chiocciole* (Fig. 13), the first book entirely devoted to shells. Its Italian edition was followed by a Latin one in 1684. Buonanni was a Jesuit priest who succeeded the Jesuit and erudite Athanasius Kircher as director of the *Kunstkammer* at *Collegium Romanum*, better known as Kircher's Museum. A remake of the same *Unio* figures appeared in *Icones testaceorum* (Fig. 14), the last part of *Musaeum Kircherianum*, a catalogue by Buonanni (1709) of the items displayed in that museum.

Lister's *Appendix ad Historiam animalium Angliae* (1681) contained a folding plate in which the author represented several British naiads (Fig. 15). One of these was a drawing of the inner side of a left valve of *M. margaritifera*, named *Musculus niger, omnium longe crassisimus, conchae longae species Gesn. Aldrov.* [black mussel, thick over its entire length, long-shelled species according to Gessner and Aldrovandi]. There also appeared the figures of two *Anodonta*, respectively named *Musculus tenuis minor* [thin small mussel] and *Musculus tenuis maximus* [thin great mussel], and two *Unio pictorum*, named *Musculus ex viridi, pallidus, omnium angustissimus* [very narrow mussel, pale green] and *Musculus angustior, ex flavo seu viridi nigricans* [narrow mussel, as often yellow as blackish green].

The monumental *Historia conchyliorum* by Lister was the first serious attempt to classify and illustrate all the testacean molluscs then known. It contained a thousand engravings after drawings by Anna and Susanne Lister, wife and daughter of this author. The first edition was printed in London in 1685–1692 and was soon followed by a second one in 1692–1697. A third, luxury edition appeared in Oxford in 1770. This was devoted to the second



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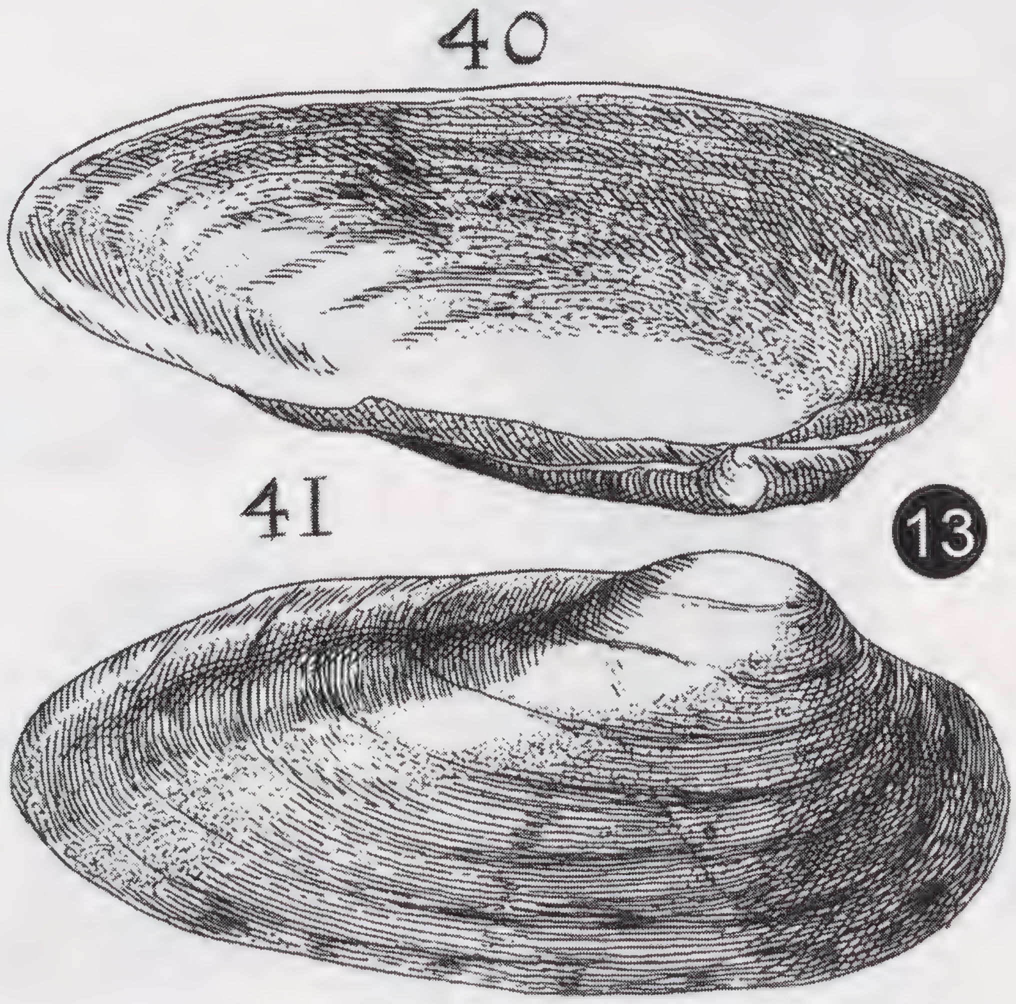
Conchæ longæ ſpecies etiam in dulcibus aquis reperitur ; cuius teſtam alteram parte eius interna

Concha longa Zoographi.



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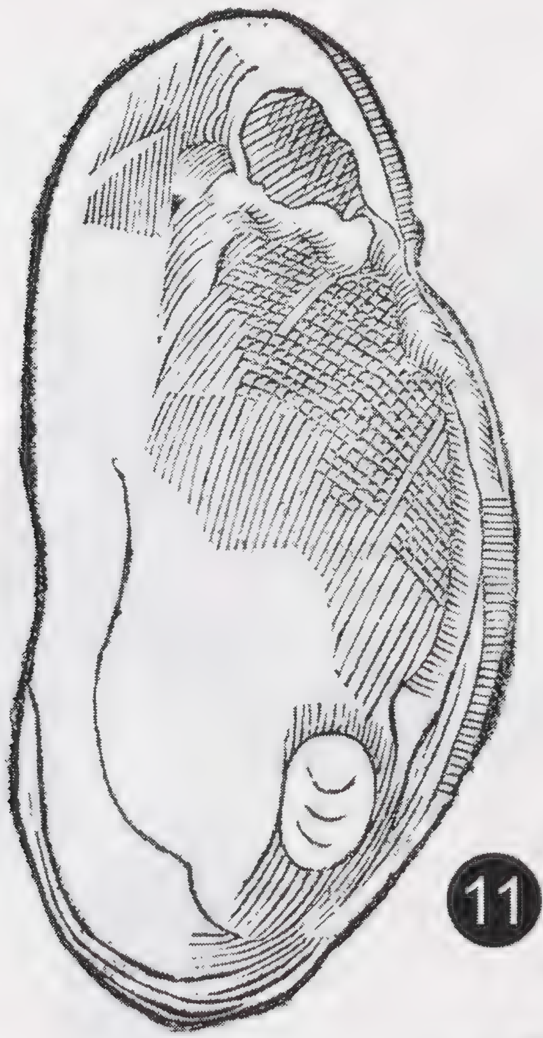

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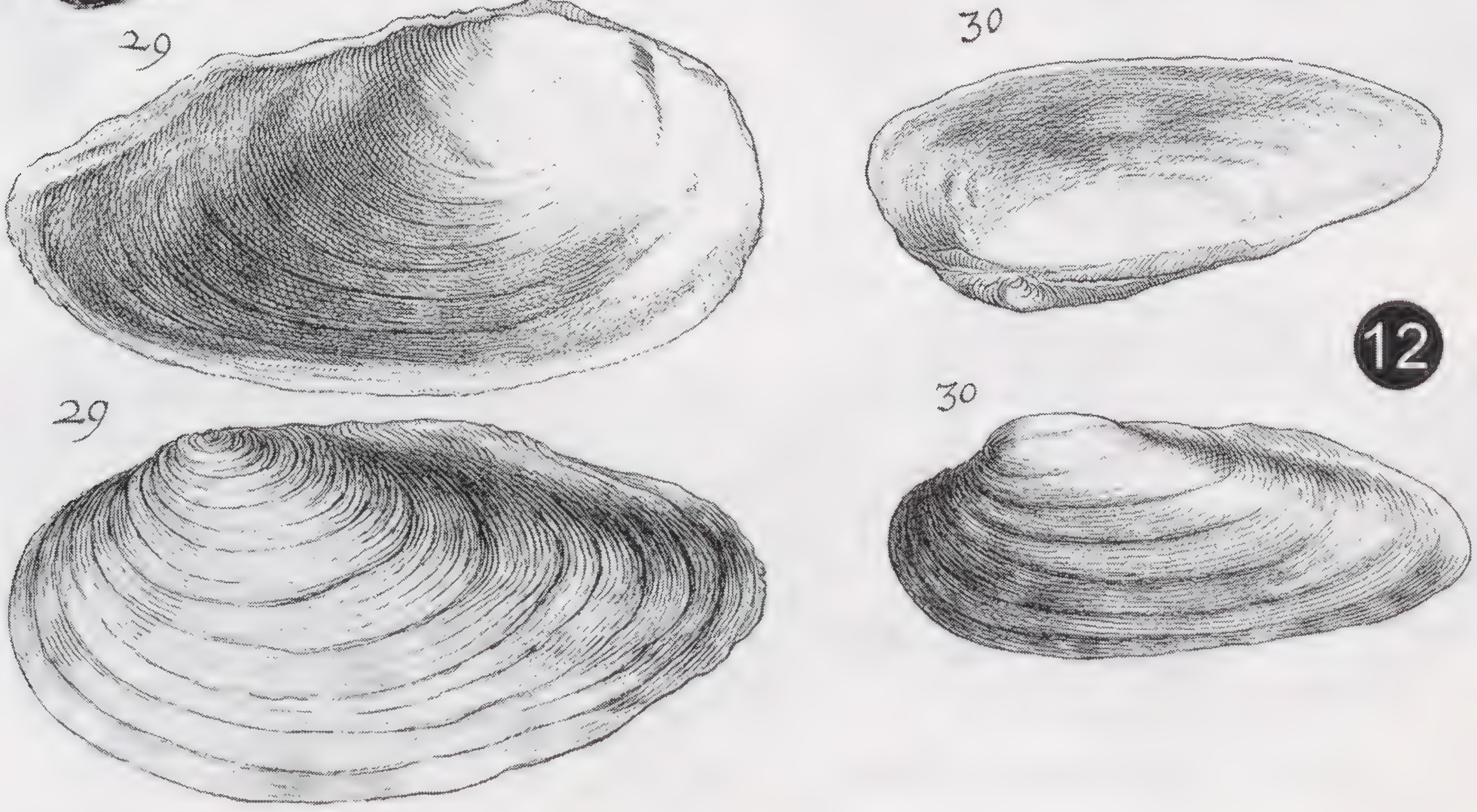

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FIGS. 10–13. FIG. 10: *Conchae longae species etiam in dulcibus aquis reperitur* from Gessner (1558); FIG. 11: *Concha longa* from Aldrovandi (1606); FIG. 12: *Musculus latus* and *Musculus angustior* from Lister (1678); FIG. 13: *Concha longa, pictorum dicta* from Buonanni (1681). FIGS. 10–12: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 13: By permission of Biblioteca Nacional, Madrid, Spain.

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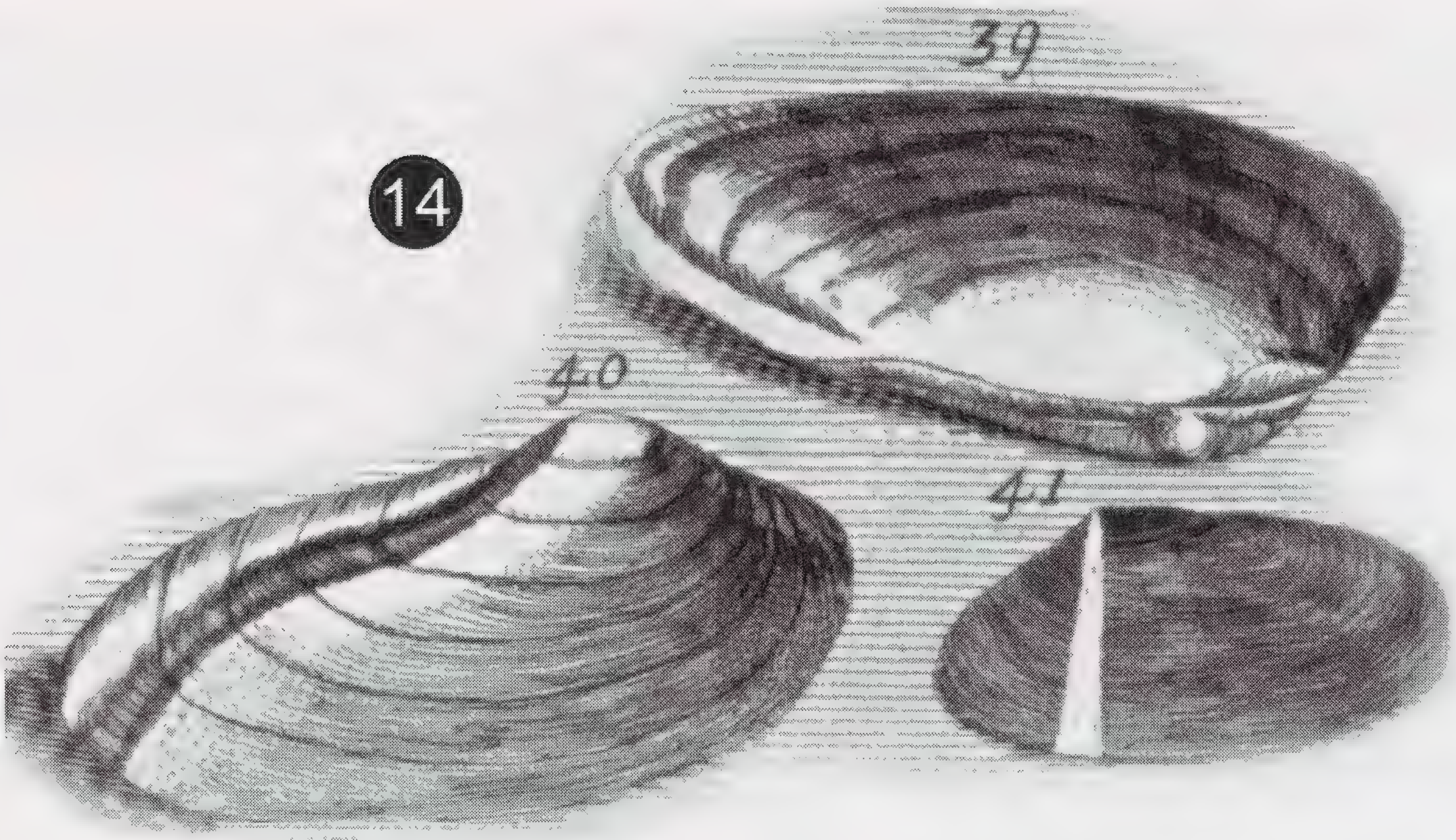

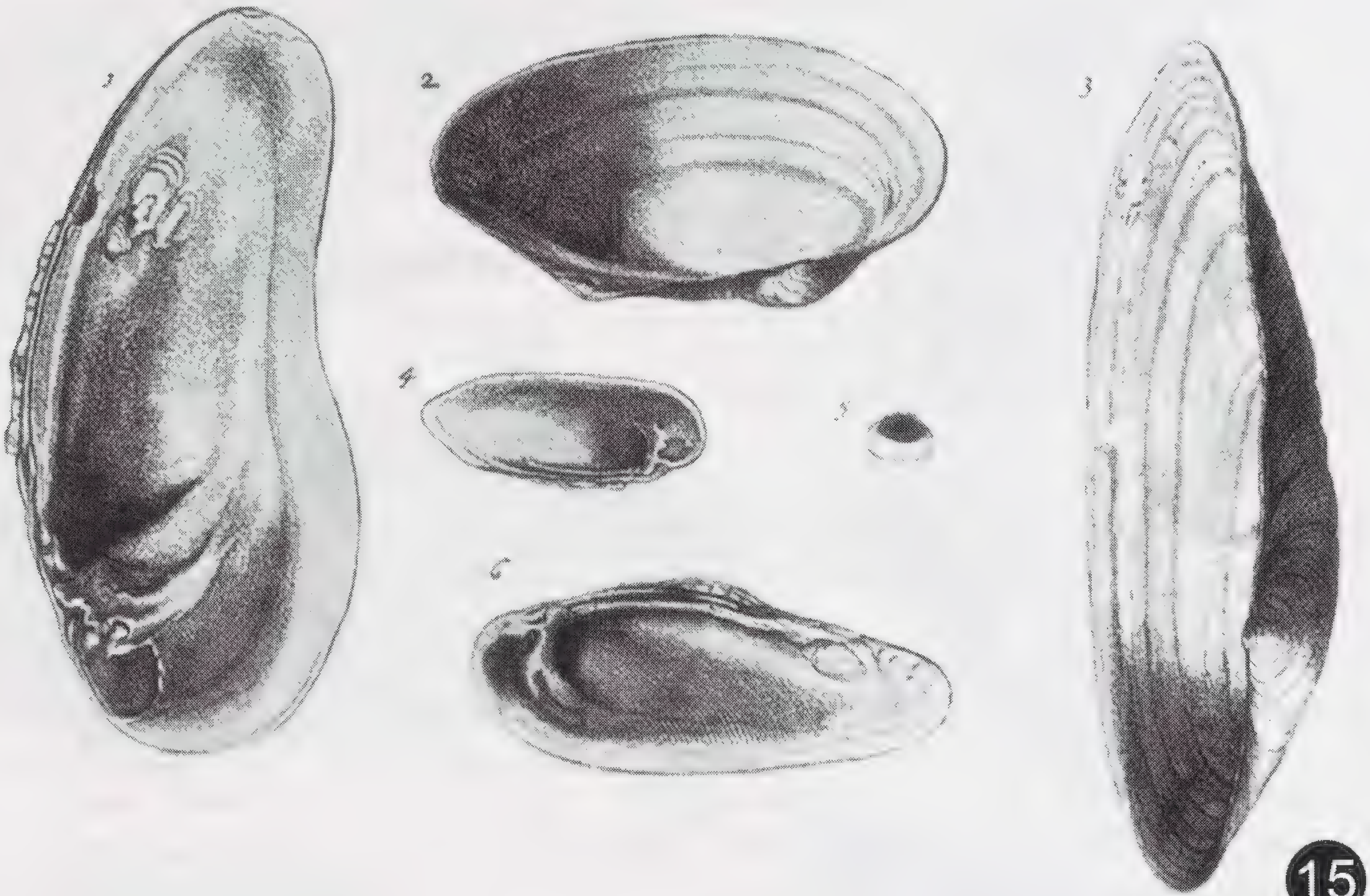

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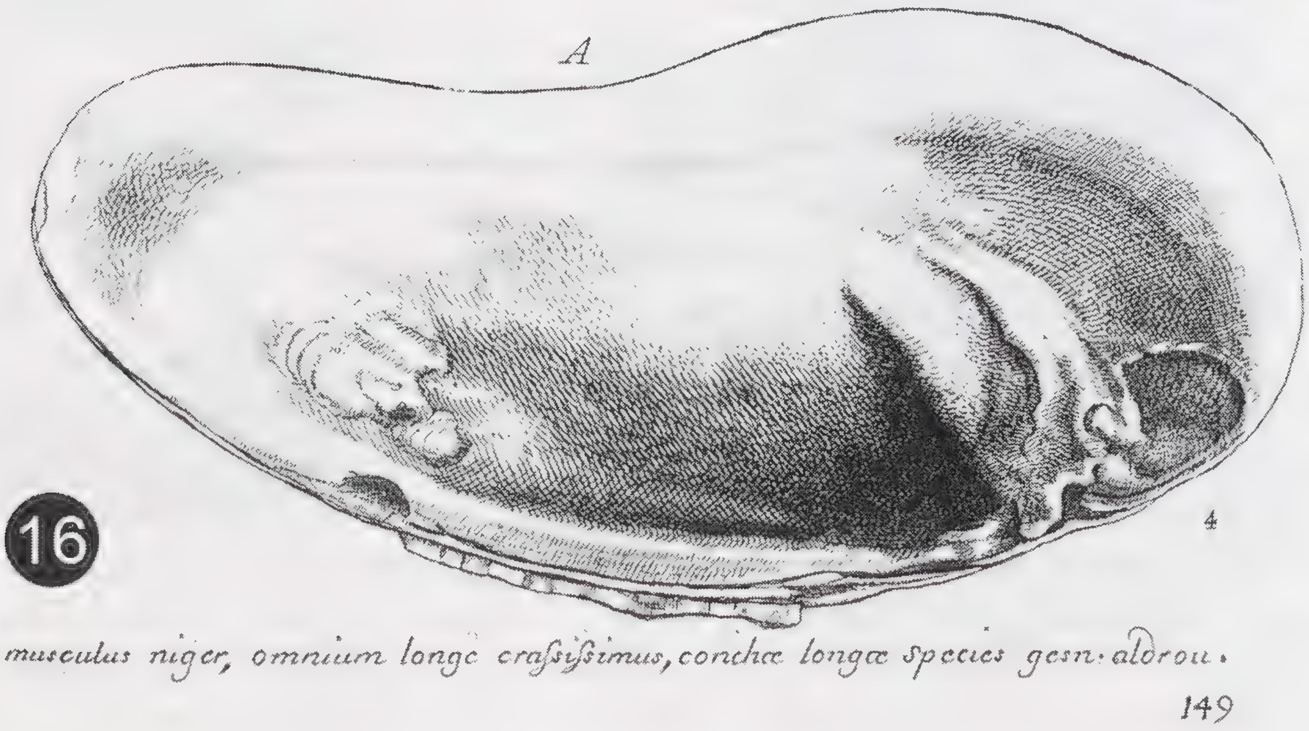
A
4
4. musculus niger, omnium longe craſsiſsimus, conchæ longæ species gesn. aldrou.
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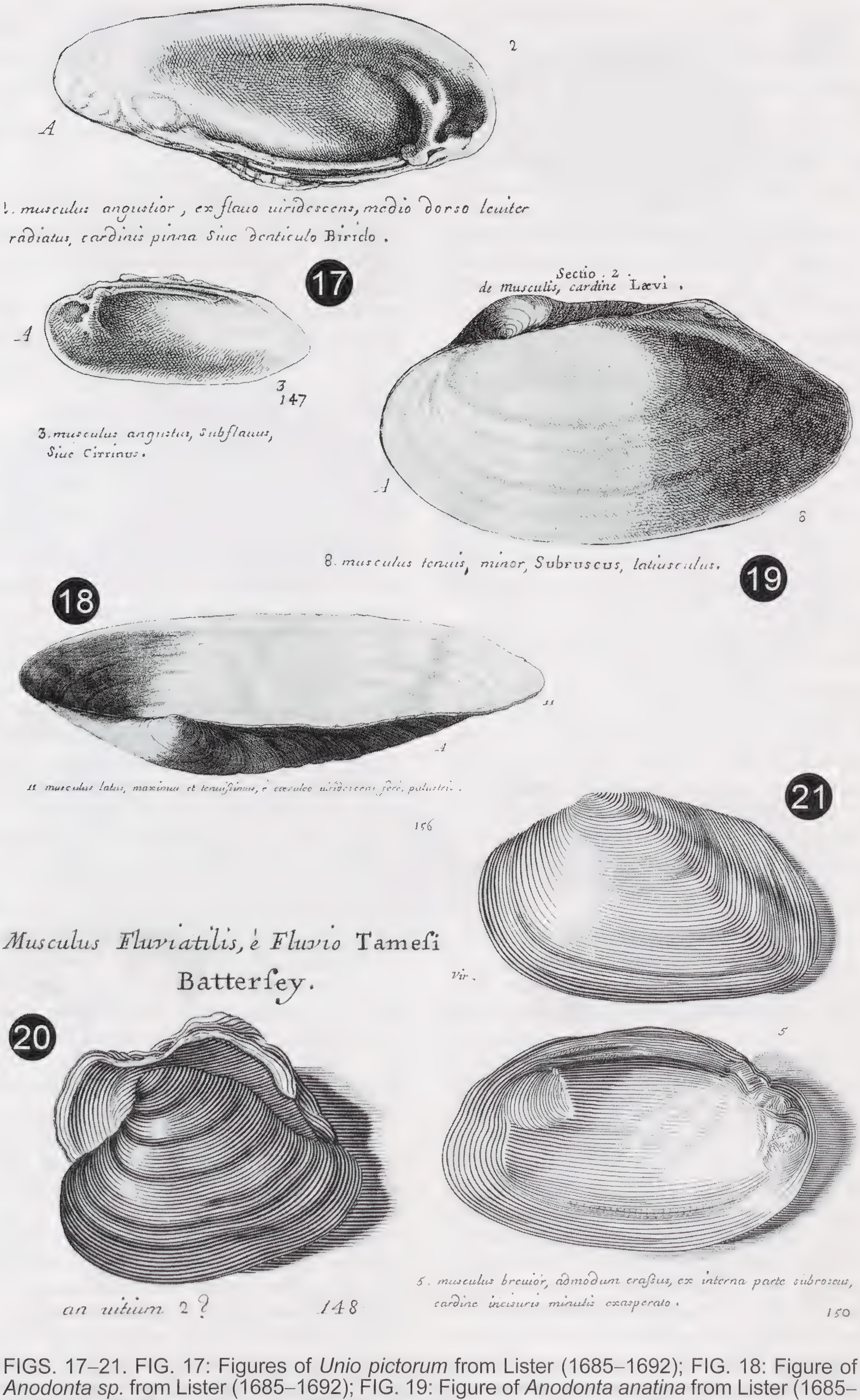
FIGS. 14–16. FIG. 14: *Concha longa, pictorum dicta* from Buonanni (1709); FIG. 15: Figures of several British freshwater mussels from Lister (1681); FIG. 16: Figure of *Margaritifera margaritifera* from Lister (1685–1692). FIGS. 14, 15: By permission of Biblioteca Nacional, Madrid, Spain; FIG. 16: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.


2
A
1. musculus angustior, ex flauo uiridescens, medio dorso leuiter radiatus, cardinis pinna siue denticulo Birido.
17
A
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147
3. musculus angustus, subflauus, siue Cirrinus.
Sectio. 2.
de musculis, cardine Lævi.
A
8
8. musculus tenuis, minor, subruscus, latiusculus.
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A
11 musculus latus, maximus et tenuiſsimus, è cœruleo uiridescens fere, palustri.
156
21
Musculus Fluviatilis, è Fluvio Tameſi Batterſey.
Vir.
20
5
5. musculus breuior, admodum craſsus, ex interna parte subroseus, cardine incisuris minutis exasperato.
150
an uitium 2?
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FIGS. 17–21. FIG. 17: Figures of *Unio pictorum* from Lister (1685–1692); FIG. 18: Figure of *Anodonta sp.* from Lister (1685–1692); FIG. 19: Figure of *Anodonta anatina* from Lister (1685–1692); FIG. 20: Figure of an unidentified freshwater mussel, probably a deformed specimen, from Lister (1685–1692); FIG. 21: Figure of an unidentified freshwater mussel from Lister (1685–1692). FIGS. 17–21: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.

Duchess of Portland, who collected shells, and was edited by William Huddesford, the curator at the Ashmolean Museum, who saved from destruction the head and one leg of the last stuffed dodo in existence. Lister bestowed on the Ashmolean his collection of British shells, naiads included, as well as the copper plates and the original drawings of *Historia conchyliorum*. In this outstanding work, he included the same figures of naiads before used (Lister, 1685) (Figs. 16–19) and added several new ones (Figs. 20–28), most species from outside England and even Europe, such as the labelled *Pectunculus subviridis, triquetrus* [little greenish, triangular shaped scallop] (Fig. 27), from the *Musaeo Oxoniensis* [Ashmolean Museum], which might be *Cristaria plicata* (Leach) from China or *Potamilus alatus* (Say, 1817) from North America. It is of interest that close to one of these figures, probably representing *Unio tumidus* Philipsson, 1788, and according to its caption *Musculus a Danubio* [mussel from the Danube River] (Fig. 28), Lister also figured what we interpret as a cord of glochidia or freshwater mussel larvae.

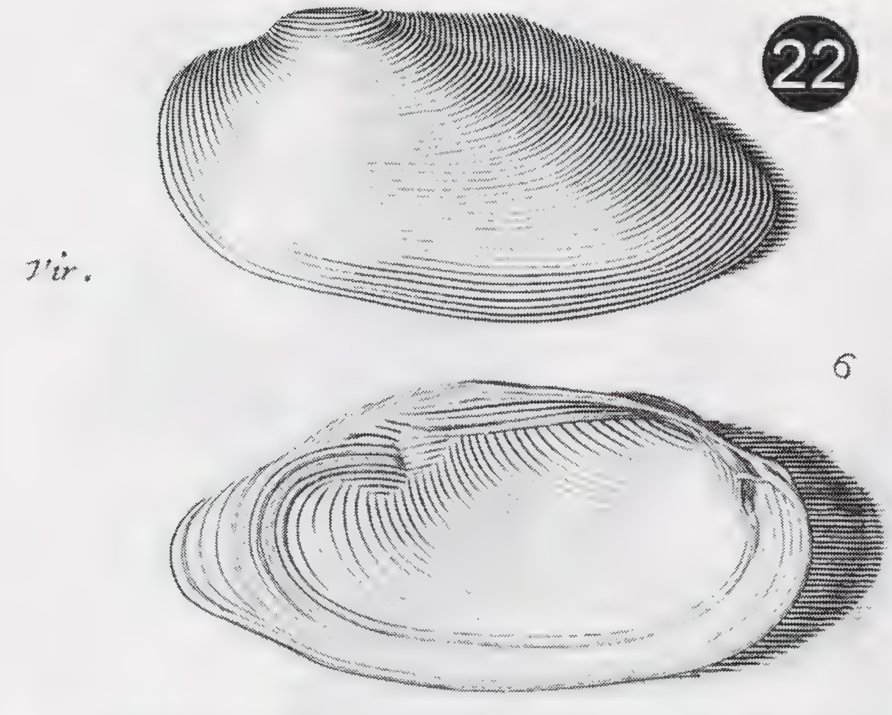
Lister (1696) was also the author of an anatomical picture of a naiad (Fig. 29). The same plate contains images of the glochidia, the ligament, and the aragonite crystals of the inner side of the shell. Nevertheless, the first author who showed the anatomy of a naiad was not Lister but Antonius de Heide (1683). This Dutch medical depicted several isolated parts of a specimen of *Anodonta* in a book that also offered the first description of the cilia motion at the gills and at the cirri around the mussel's mouth (Heard, 1998).

The Dutch Antoni van Leeuwenhoek (1697), who with Heide and Buonanni was an early microscopist, illustrated several naiads (Fig. 30). This work was a sequel to Leeuwenhoek (1695), a collection of studies on microscopy observations written as illustrated letters, most of them dedicated to the Royal Society. The represented species is *Anodonta cygnea* showing its soft parts, eggs and glochidia, which Leeuwenhoek called *Pisciculus testaceus* [small clams]. This author was the first to distinguish the sex of freshwater mussels based on observations of eggs and spermatozoids in different specimens. He also observed eggs passing to the gills and even tried to follow the development of larvae when they were removed from the gills, yet these died shortly after their isolation. The metamorphosis of glochidia into juvenile mussels as parasites of fishes was discovered much later by Houghton (1862) and Leydig (1866). Before these authors, Rathke (1797) wrongly concluded that glochidia were animals that parasitize the gills of fishes and assigned them the spurious specific name of *Glochidium parasiticum* (Heard & Dinesen, 1999).

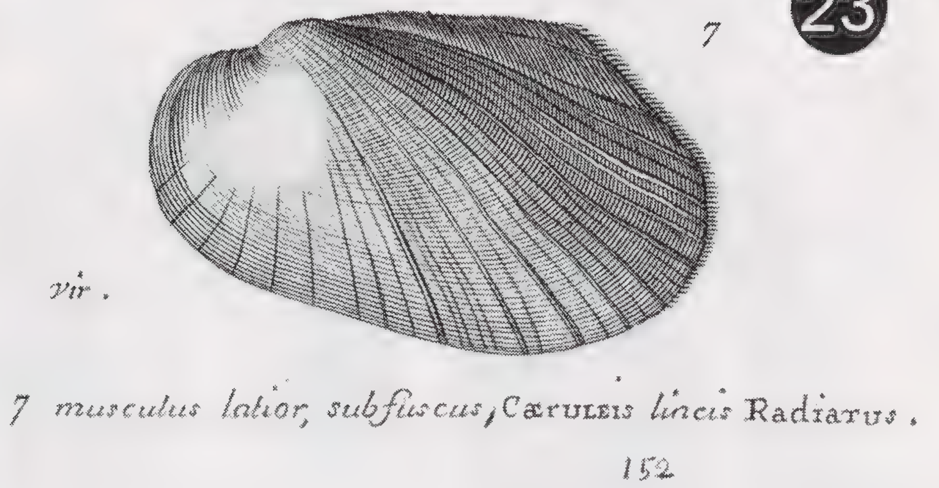
The first living naiad in its own environment was figured by Poupart (1731) (Fig. 31B), who wrote the first natural history of freshwater mussels. This figure was later copied by Scheuchzer (1735). The image shown is one of a semiburied naiad specimen moving forward to create a typical track or furrow in the sediment. Poupart (1731) also depicted two valves of *Anodonta anatina*, a dorsal view of the shell and a detail of its ligament (Fig. 31A).

The next chronological illustration is a curious and anonymous poster kept at the cloister of Ebstorf, Germany (Fig. 32). The poster is dated 1736 and was created for standing at the banks of the Schwienau River in High Saxony. The warning of the more than unpleasant results of violating the prohibition of fishing for freshwater mussels is clearly expressed by an axe, a sectioned left hand and a specimen of *M. margaritifera* with its valves open showing three pearls inside. The legend says *Hüte Dich fur Schaden* [preserve your hands]. Another anonymous illustration related to pearl fishing in High Saxony is a drawing dated *circa* 1760 and entitled *Perlen-Sucher in seinem Wasser-Habit* [pearl searcher in his water outfit] (Fig. 33). The subject, who is accompanied by a poodle or water dog, has a pearl mussel in his left hand while in the right carries a large wooden forceps to collect submerged naiads. He also carries a musket on his back for hunting and protection from bears, which were then more abundant than now along the Paleartic waterways inhabited by *M. margaritifera.* At this point, mention should be made of a pencil sketch by Linnaeus. The drawing (Fig. 34) appeared in *Iter Lapponicum* or *Journey to Lapland*, a hand-written account of his trip to Lapland in the summer of 1732. The journal was found in 1953 during the repair of the building at the botanical gardens of the University of Uppsala, where Linnaeus lived for over 30 years. The sketch of a pearl fishery at a place called Purkijaur depicts a man on a simple raft anchored to the bottom fishing for freshwater mussels using a large forceps. Close to him is outlined a specimen of *M. margaritifera*. In the related text, Linnaeus reflects on the idea of inventing a method to induce the formation of pearls in freshwater

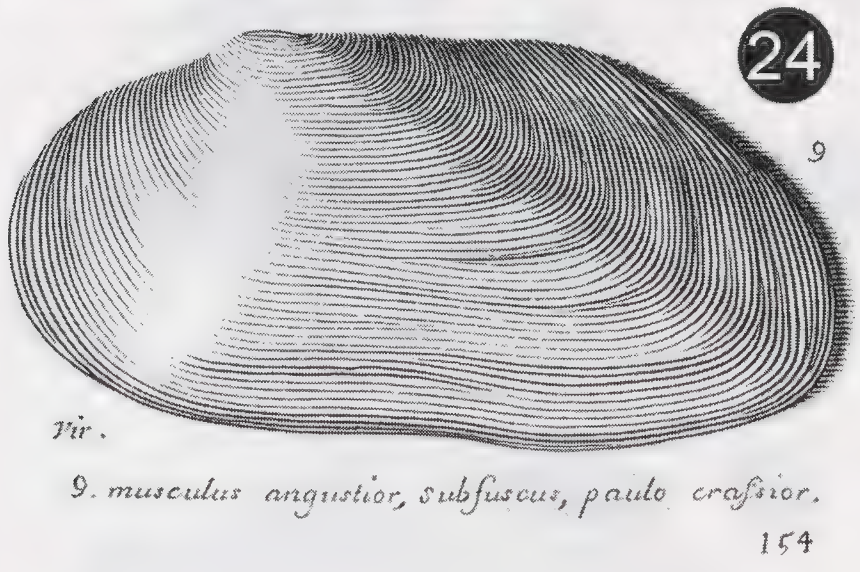
22


Vir.
6
6. musculus fuscus, angustior, ex altera parte Cuneatim protensus.

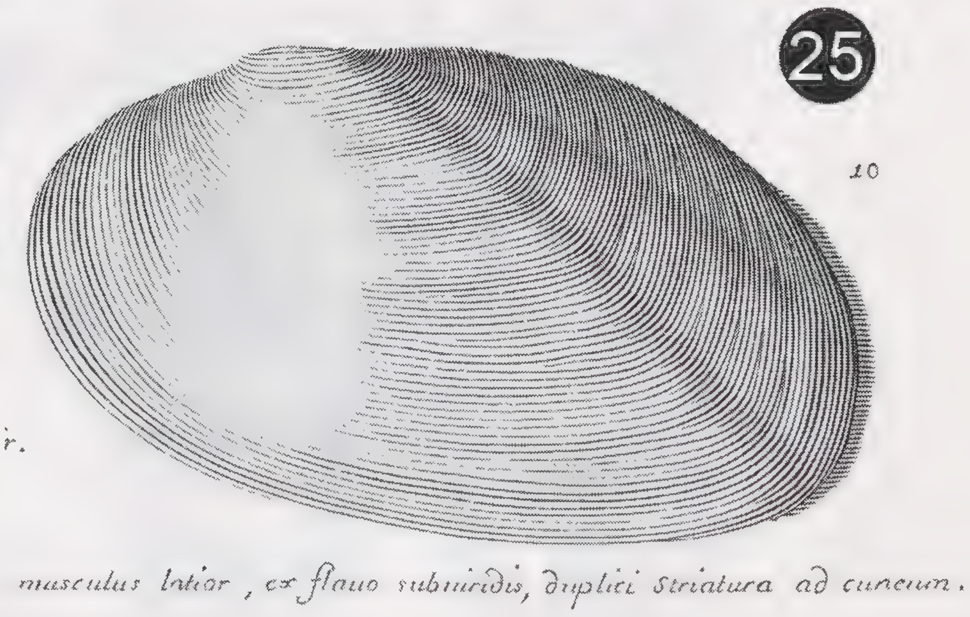
23


7
vir.
7 musculus latior, subfuscus, Cæruleis lineis Radiatus.
152

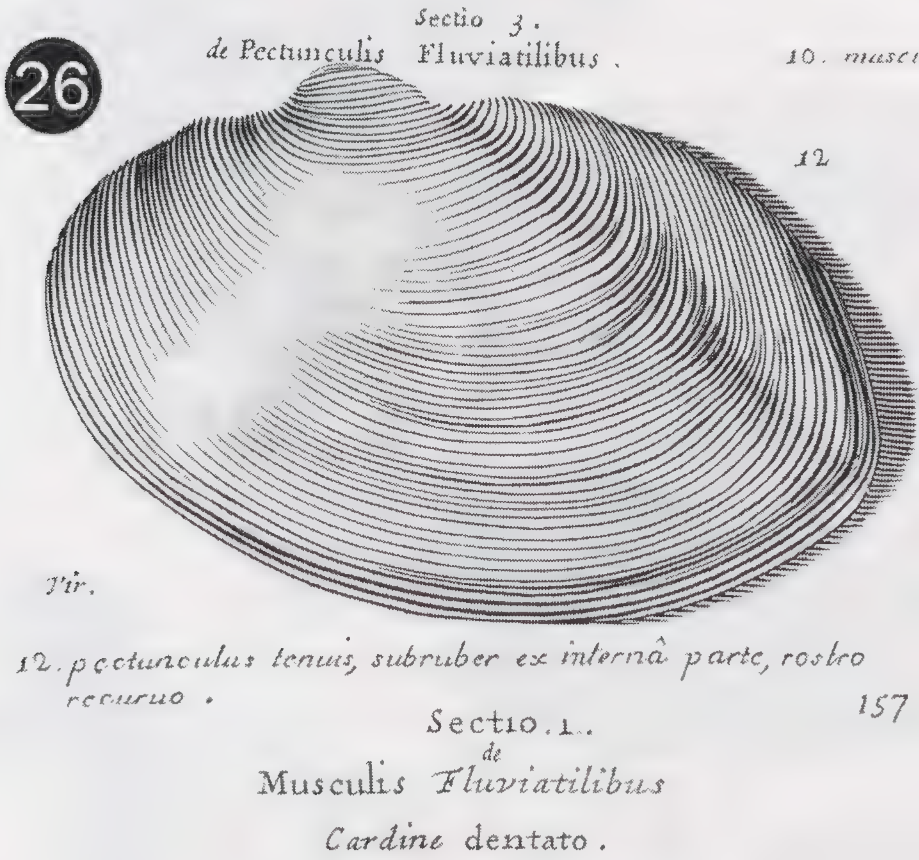
24


9
Vir.
9. musculus angustior, subfuscus, paulo crassior.
154

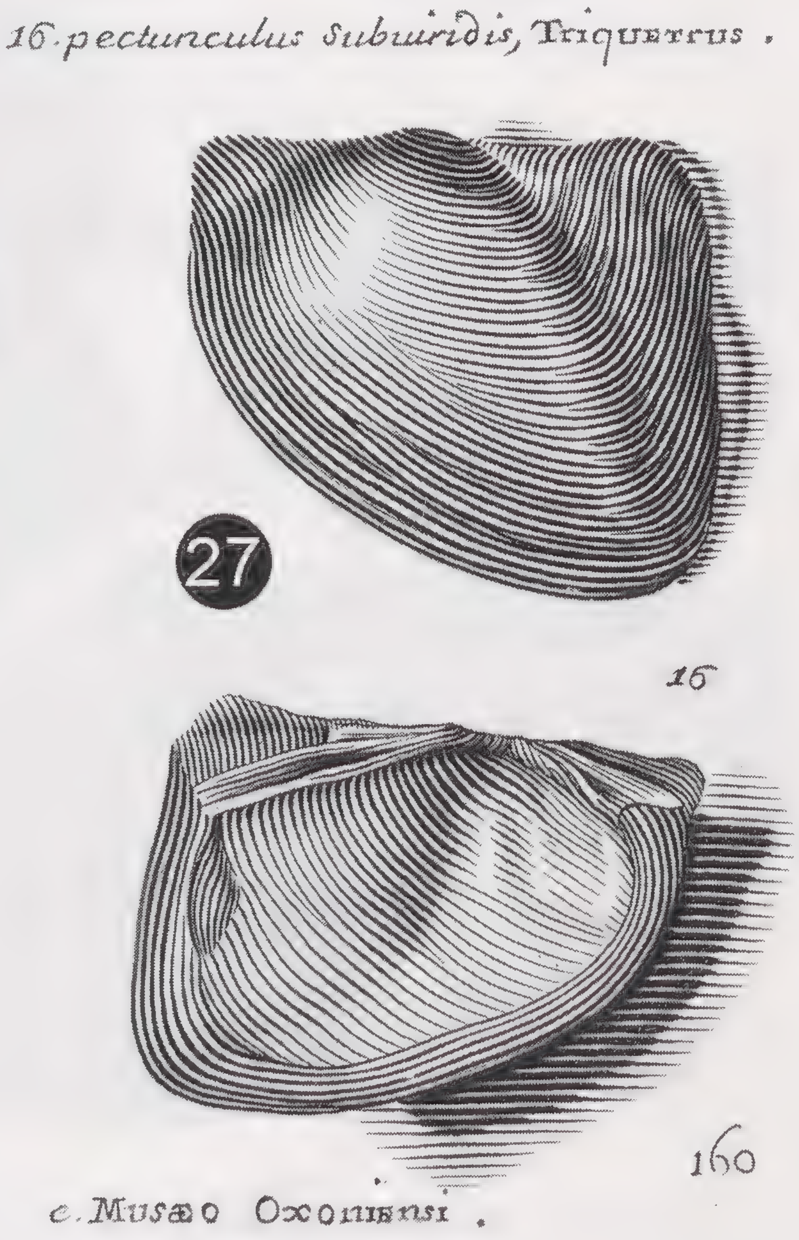
25


10
r.
10. musculus latior, ex flauo subuiridis, duplici striatura ad cuneum.

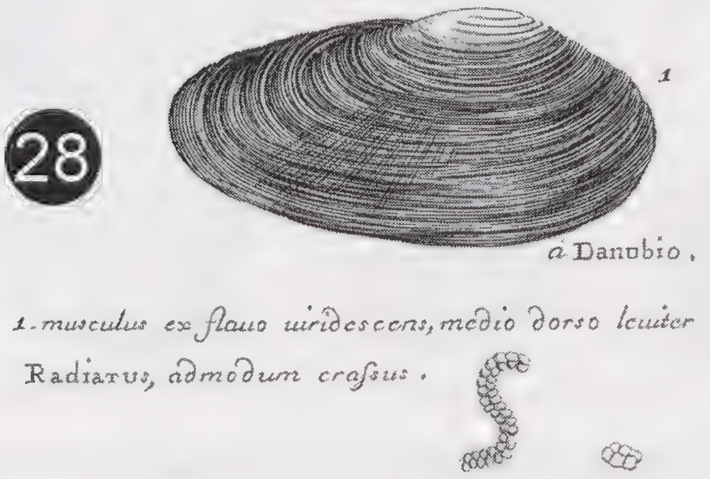
26


Sectio 3.
de Pectunculis Fluviatilibus.
12
Vir.
12. pectunculus tenuis, subruber ex internâ parte, rostro recurvo.
157
Sectio. 1.
de
Musculis Fluviatilibus
Cardine dentato.

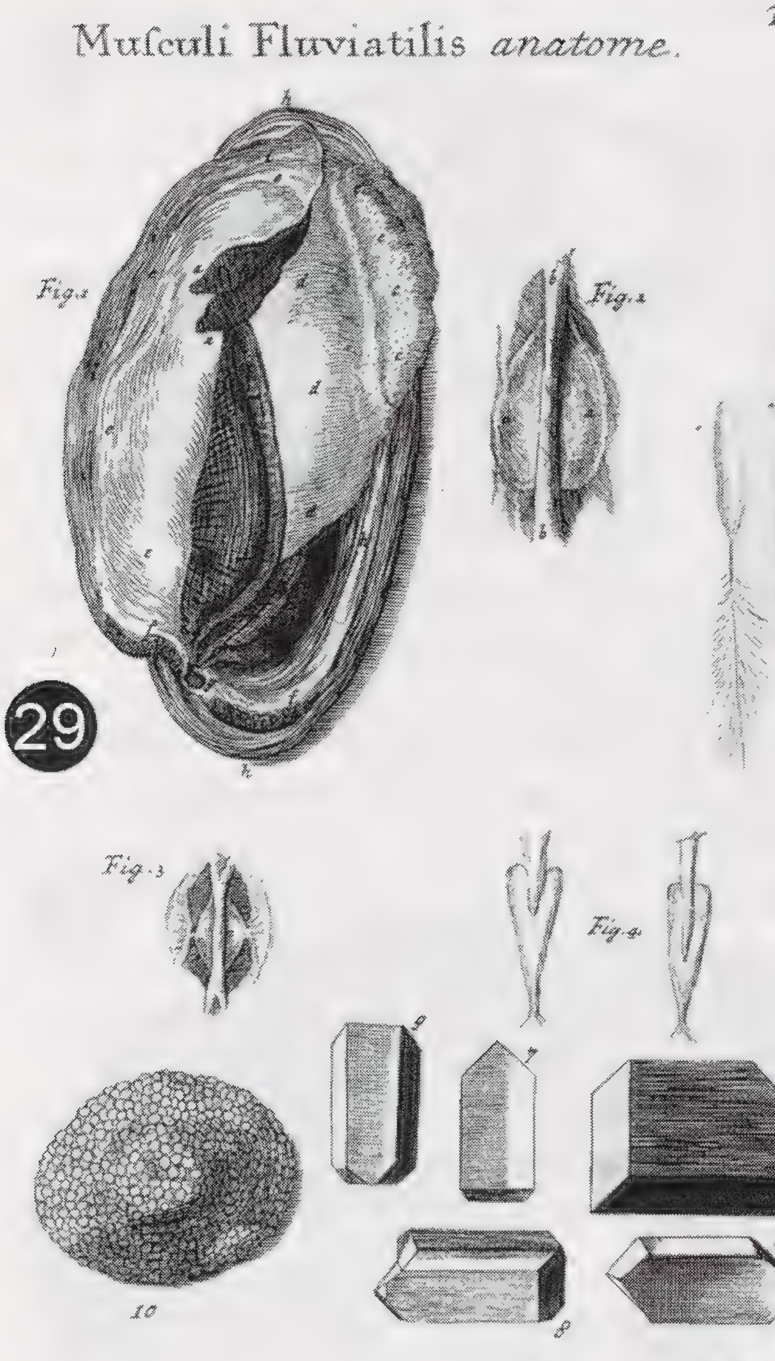
27


16. pectunculus Subuiridis, Triquetrus.
16
160
e. Musæo Oxoniensi.

28


1
a Danubio.
1. musculus ex flauo uiridescens, medio dorso leuiter Radiatus, admodum crassus.

FIGS. 22–28. FIGS. 22–26: Figures of several unidentified freshwater mussels from Lister (1685–1692); FIG. 27: Figure of an exotic freshwater mussel from Lister (1685–1692); FIG. 28: Figure of *Unio tumidus* and glochidia from Lister (1685–1692). FIGS. 22–28: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.



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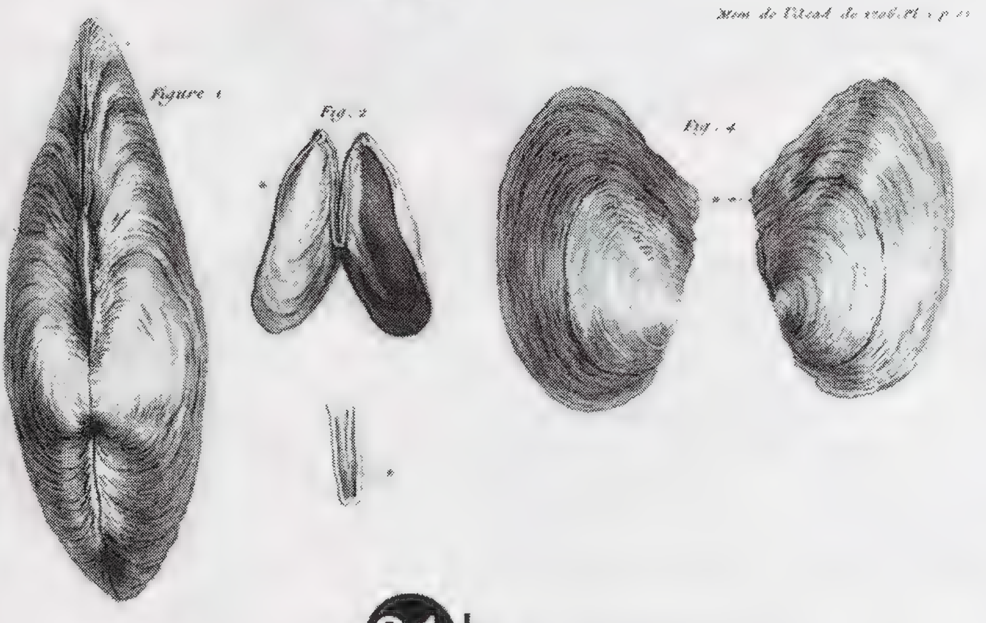
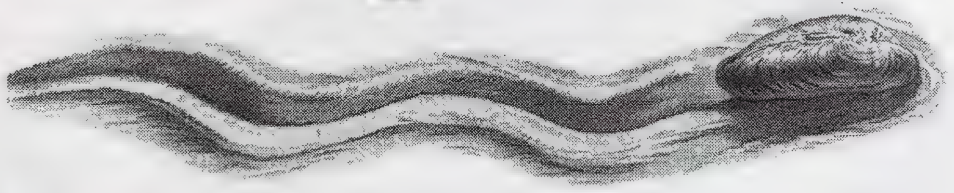
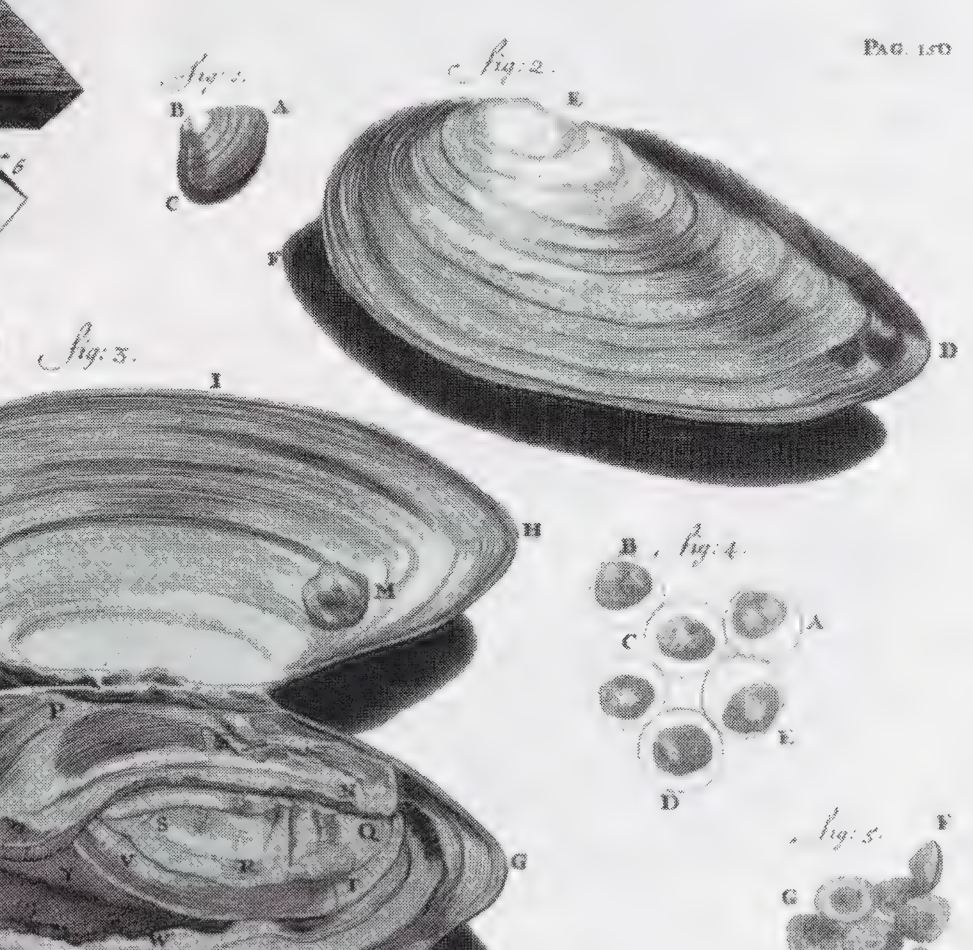


31a



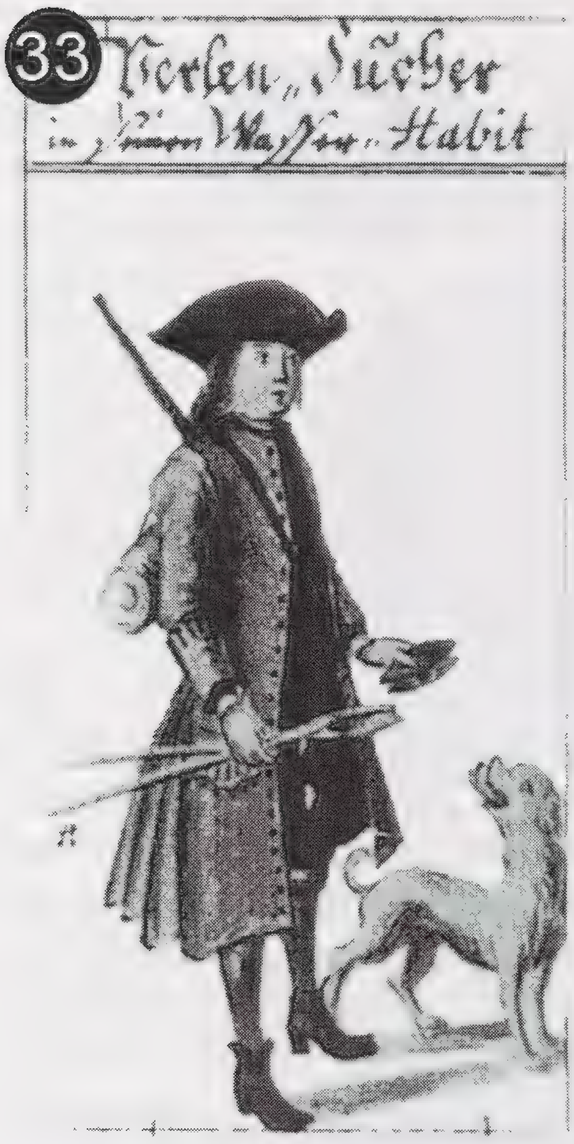
31b

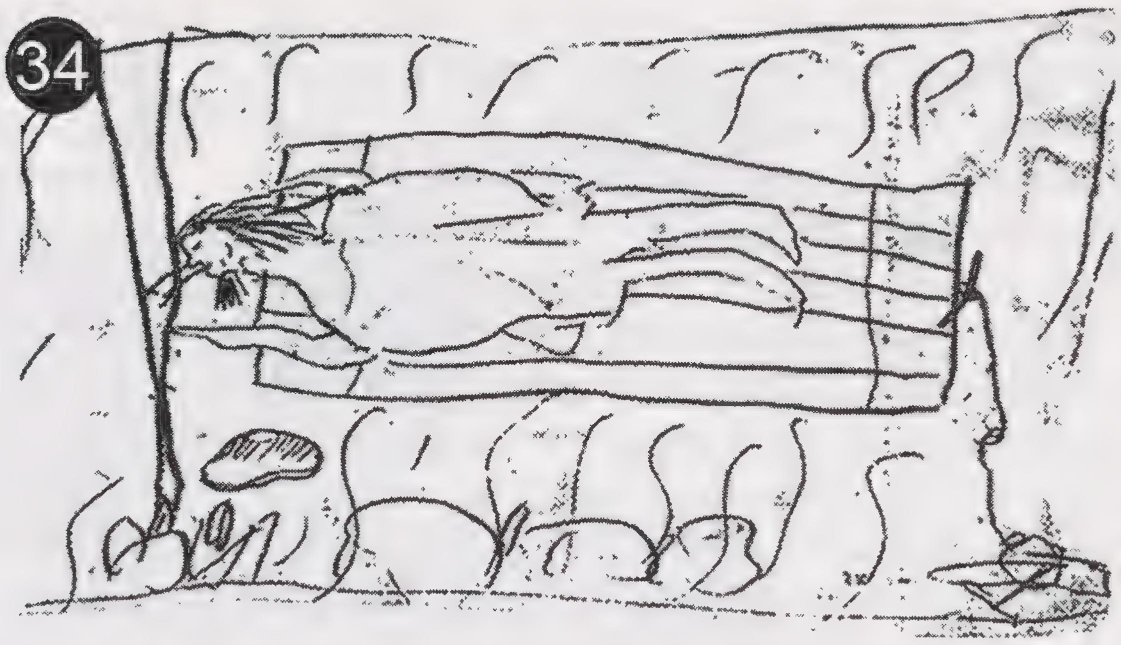


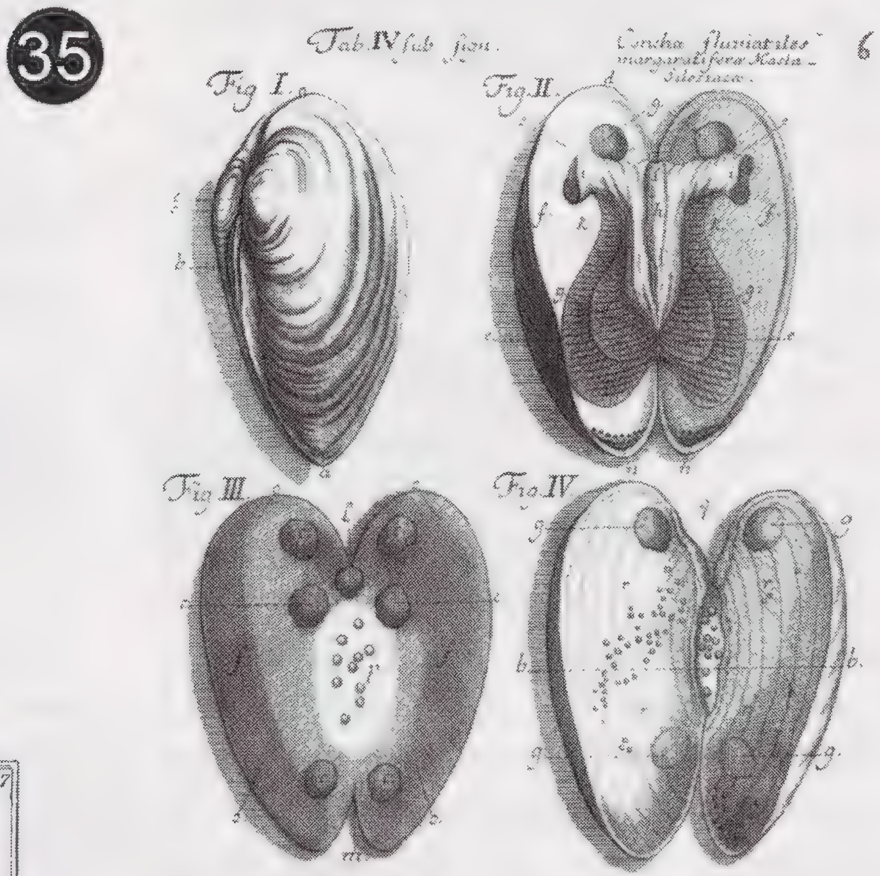
30

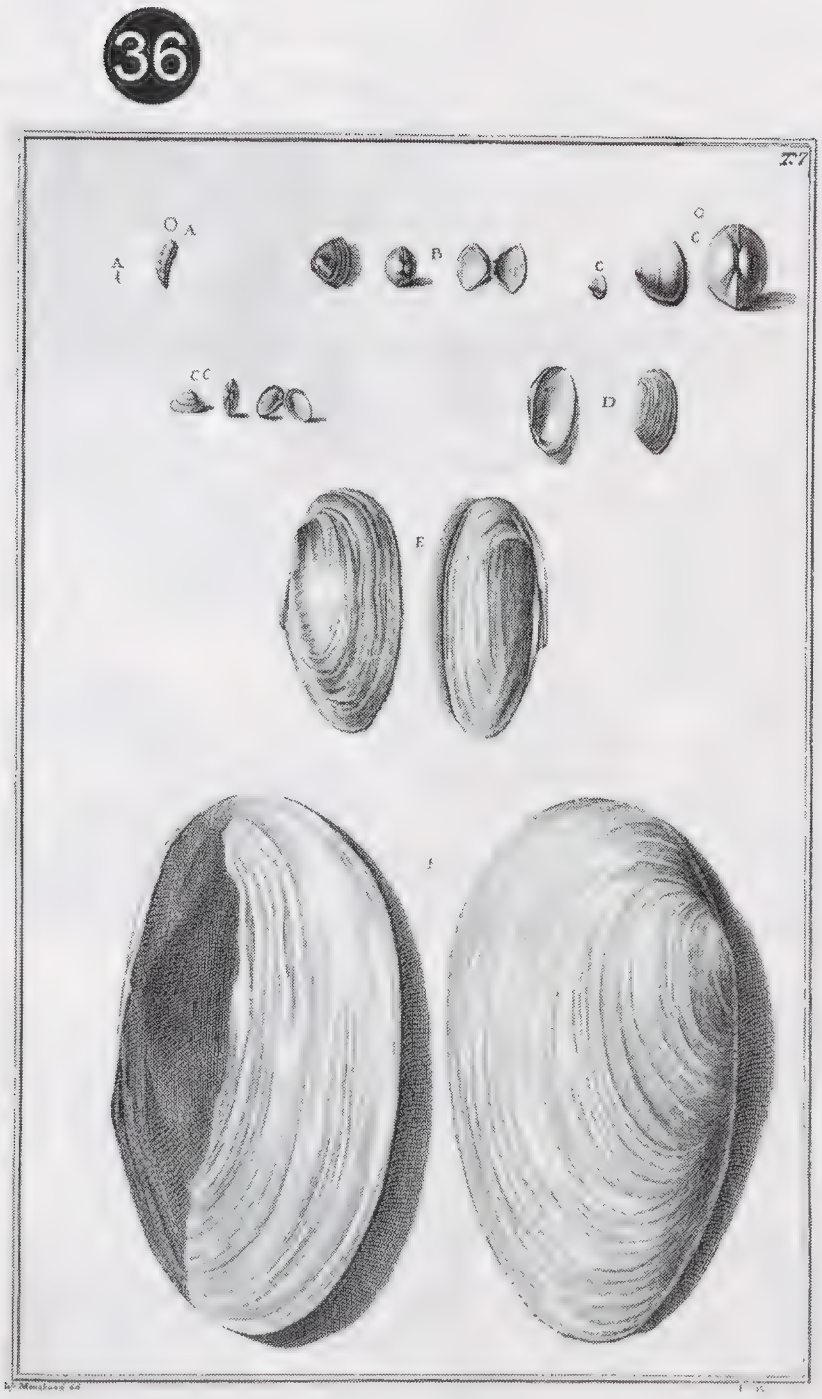
32

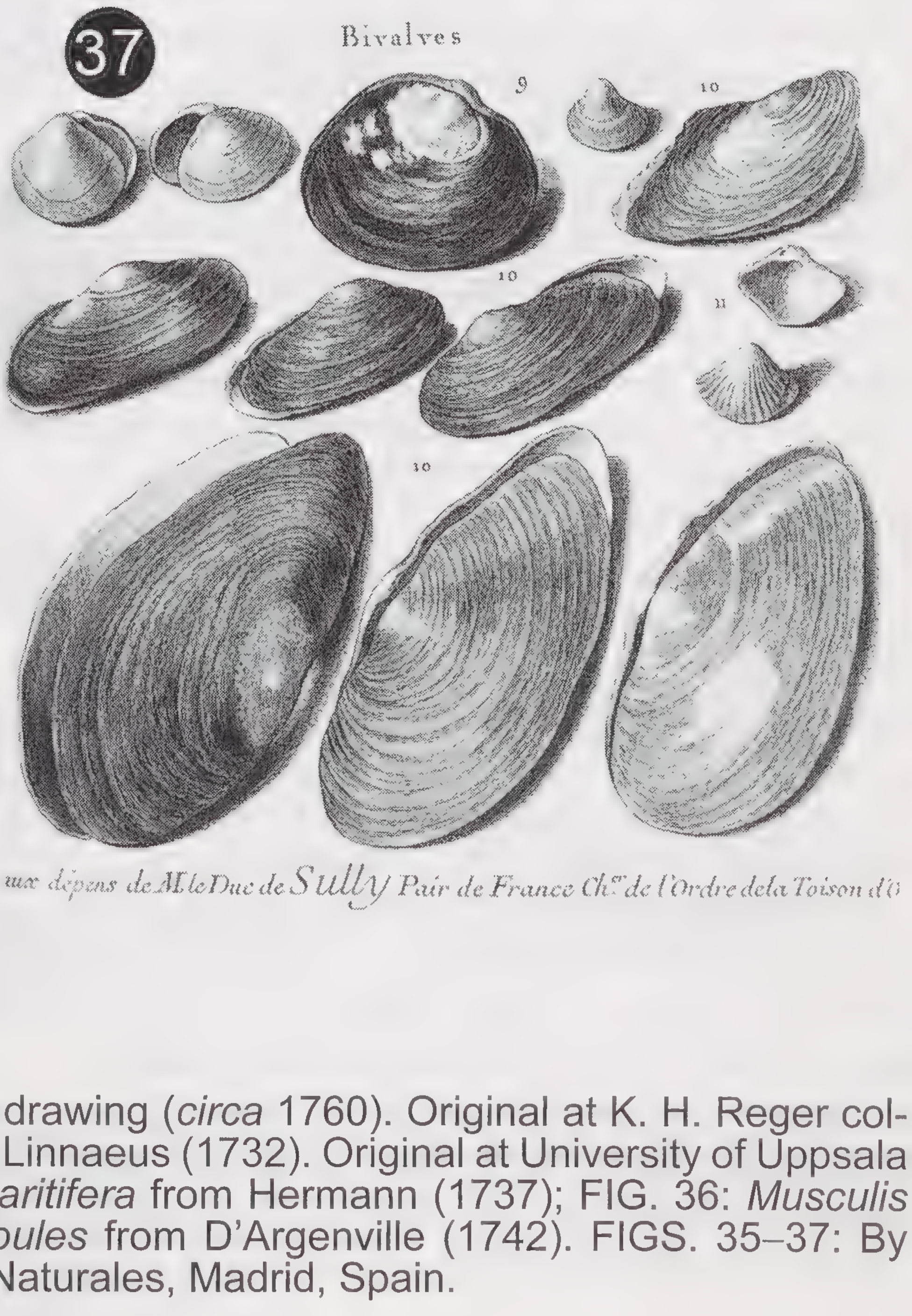
FIGS. 29–32. FIG. 29: External anatomy of a freshwater mussel and images of its glochidia, ligament and aragonite crystals of the shell from Lister (1696); FIG. 30: Images of a freshwater mussel, its soft parts, eggs and glochidia from Leeuwenhoek (1697); FIG. 31 A: *Moule* from Poupart (1731); B: Freshwater mussel showing its track in the sediment from Poupart (1731); FIG. 32: German anoymous poster (1736). Original at Kloster Ebstorf (Germany). FIGS. 29, 30: By permission of Biblioteca Nacional, Madrid, Spain; FIG. 31: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.






35
Tab. IV
Concha fluviatiles margaritifera
6
Fig. I.
Fig. II.
Fig. III.
Fig. IV.




37
Bivalves
9
10
10
11
10
aux dépens de M. le Duc de Sully Pair de France Chev. de l'Ordre de la Toison d'O

FIGS. 33–37. FIG. 33: German anonymous drawing (*circa* 1760). Original at K. H. Reger collection (Germany); FIG. 34: Pencil sketch by Linnaeus (1732). Original at University of Uppsala (Sweden); FIG. 35: *Concha fluviatiles margaritifera* from Hermann (1737); FIG. 36: *Musculis fluviatilis* from Gualtieri (1742); FIG. 37: *Moules* from D'Argenville (1742). FIGS. 35–37: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.

mussels after a comment that the fishery at Purkijaur had been practically depleted, with thousands of mussels unnecessarily killed because there was no way of knowing if they contained pearls or not.

In the 18th century, a number of opuscules and disquisitions speculating on the origin of pearls and their nature were published. Most of these lacked any drawings of naiads or any illustrations. An exception is one by a Leonhard David Hermann (1737) on the freshwater pearl shells of Silesia (Fig. 35). Four different images appear representing the inner and outer sides, the soft parts and the pearls of *M. margaritifera*.

The Florentine physician and naturalist Nicoló Gualtieri (1742), physician to Duke Cosimo III of Medici and a shell collector, figured several specimens of *Anodonta* and *Unio* (Fig. 36). That same year saw the publication of the first edition of *La conchyliologie* by Antoine Joseph Dezallier D'Argenville (1742), a councilman of King Louis XV of France who also collected shells. Several figures of naiads are there included (Fig. 37). According to the text, most of them were from the French rivers Gobelins, Loire, Marne, and Seine, but others seemed to be from North America. The second and third editions of this book, published in 1757 and 1780, were different. They added an anatomy section in which three specimens of *Unio* and *Anodonta* showing their feet were figured (Fig. 38). The third edition, post-Linnean in age, was prepared by the father and son editors Favanne de Montçervelle and included a beautiful plate with 30 figures of naiads symmetrically positioned in Rococo style (Fig. 39).

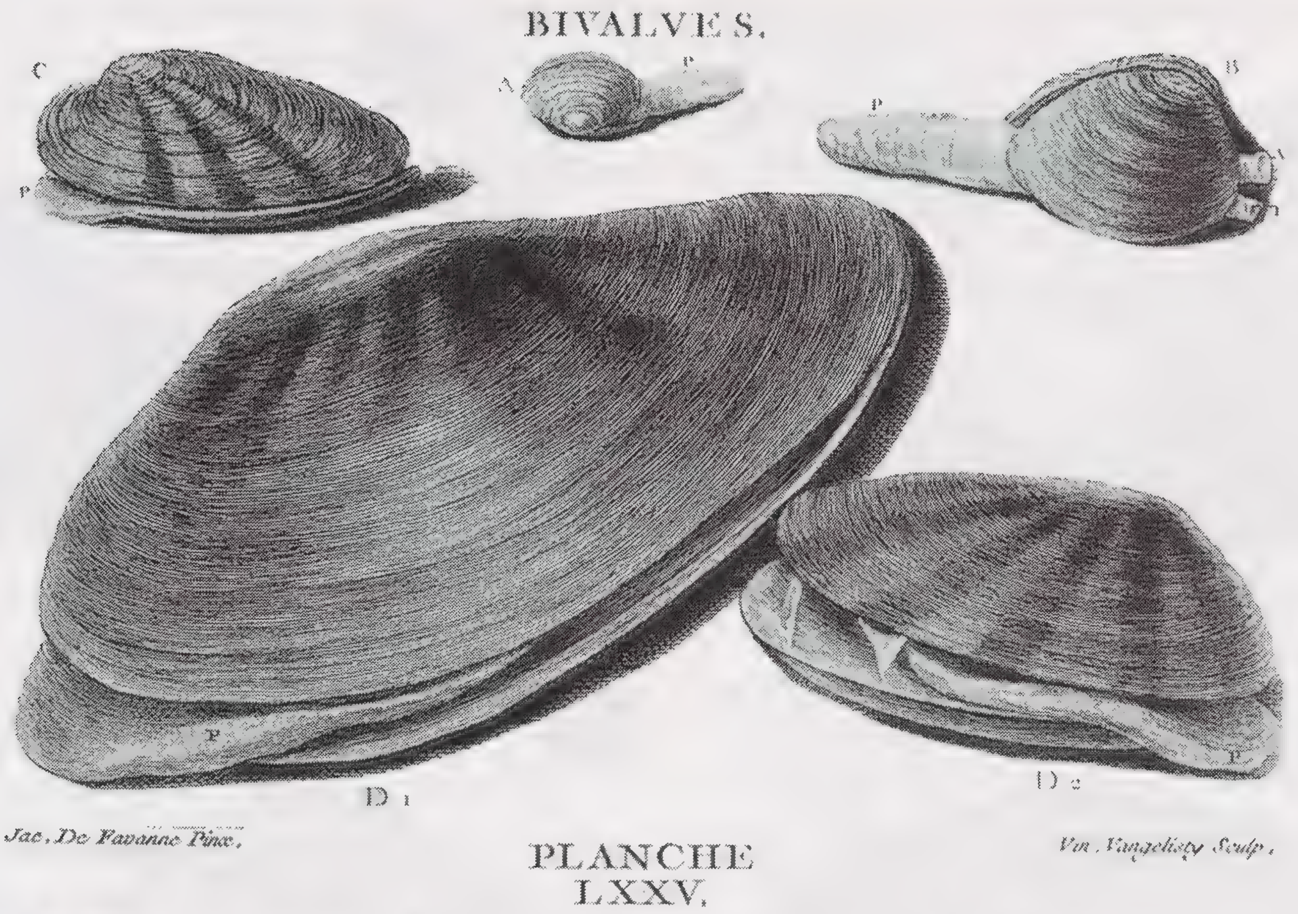
Images of naiads abide in publications of the second half of the 18th century. An illustration of *Unio* by the Dutch physician and entomologist Jan Swammerdam (1737) appeared in *Bybel der Natuure* (Fig. 40). This illustration only showed the anatomy of the species, as expressed by its caption, which says *Mytuli concha, interna representata* [shell of mussel internally depicted]. Another *Unio* was figured by the Earl Luigi Ferdinando Marsigli (1744) in the second volume of his *Description du Danube* (Fig. 41), a luxury encyclopaedia on the Donau River. Identical to the pictures of *Margaritifera* and *Anodonta* by Lister (1685, 1685–1692) were those figured by Jakob Theodor Klein (1753). This author, secretary of the Senate of Danzig, Germany, respectively named them *Diconcha sulcata* (Fig. 42) and *Musculus latus* (Fig. 43). Another *Unio* (Fig. 44) was figured among several marine bivalves by Frans Valentijn (1754), who was a priest at the Dutch colony in Ambon, Moluccas, and wrote a supplement to *D'Amboinsche Rariteitkamer* by Rumpf (1705). Erik Ludwigsen Pontoppidan (1755), bishop of Bergen, Norway, depicted *M. margaritifera* with two pearls (Fig. 45). Several naiads of the genera *Anodonta* and *Unio*, designated *Conca de Pittori* (Figs. 46, 47), were depicted by the Earl Giuseppe Ginanni (1757), also including a representation of glochidia. Finally, an image of an African freshwater mussel belonging to the genus *Mutela* (Fig. 48) was figured by Michael Adanson (1757). This author refers to the drawing as *Le mutel* and mentions it was fished in the lakes of Senegal.

The last illustration of a naiad we considered is also the first coloured one (Fig. 49). It appears in Georg Wolfgang Knorr's *Vergnügen der Augen und des Gemüths*, a book printed in 1757–1773, its French version edited in 1760–1773 as *Les délices des yeux et de l´esprit*, paraphrasing Buonanni (1681). Knorr worked as an engraver in Nuremberg, Germany. From a purist perspective, we must say this nice drawing of a naiad, water-coloured by hand and clearly inspired by the pictures by Gessner (1558) and Lister (1685, 1685–1692), is ten years post-Linnean because the fourth part of the book by Knorr, where it is, was not printed till 1769. The figure depicted is the inner side of a left valve of *M. margaritifera* showing the hinge, the pallial line, the two adductor muscle scars and the reflections from the nacre. Its caption says *La moule a perles de l'Elster* [the pearl mussel from the Elster River] and it adds *Ex Museo Mülleriano* [from Müller's collection].

ILLUSTRATION OF LIVING FRESHWATER MUSSELS, THEIR SOFT PARTS, EGGS AND GLOCHIDIA

As we have seen, living naiads or details of their anatomy were scarcely depicted in pre-Linnean times (Table 1). Adanson (1757) criticized shell collectors for their lack of scientific interest in the study of molluscs, which he pointed out to be humble animals in their external aspect but able to construct such beautiful shells, noting that conchology can be only viewed as one aspect of malacology: "The same beauty of shells that induced the interest for them has become a serious obstacle for the progress of natural history".

38



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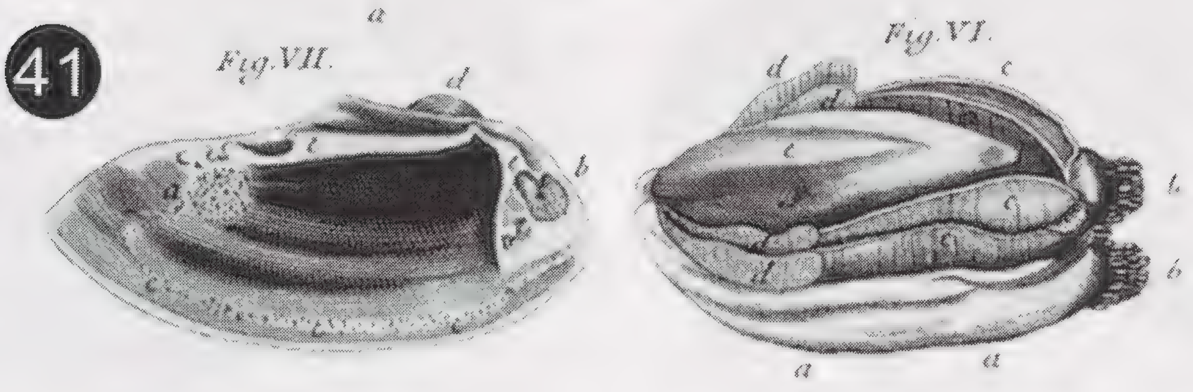

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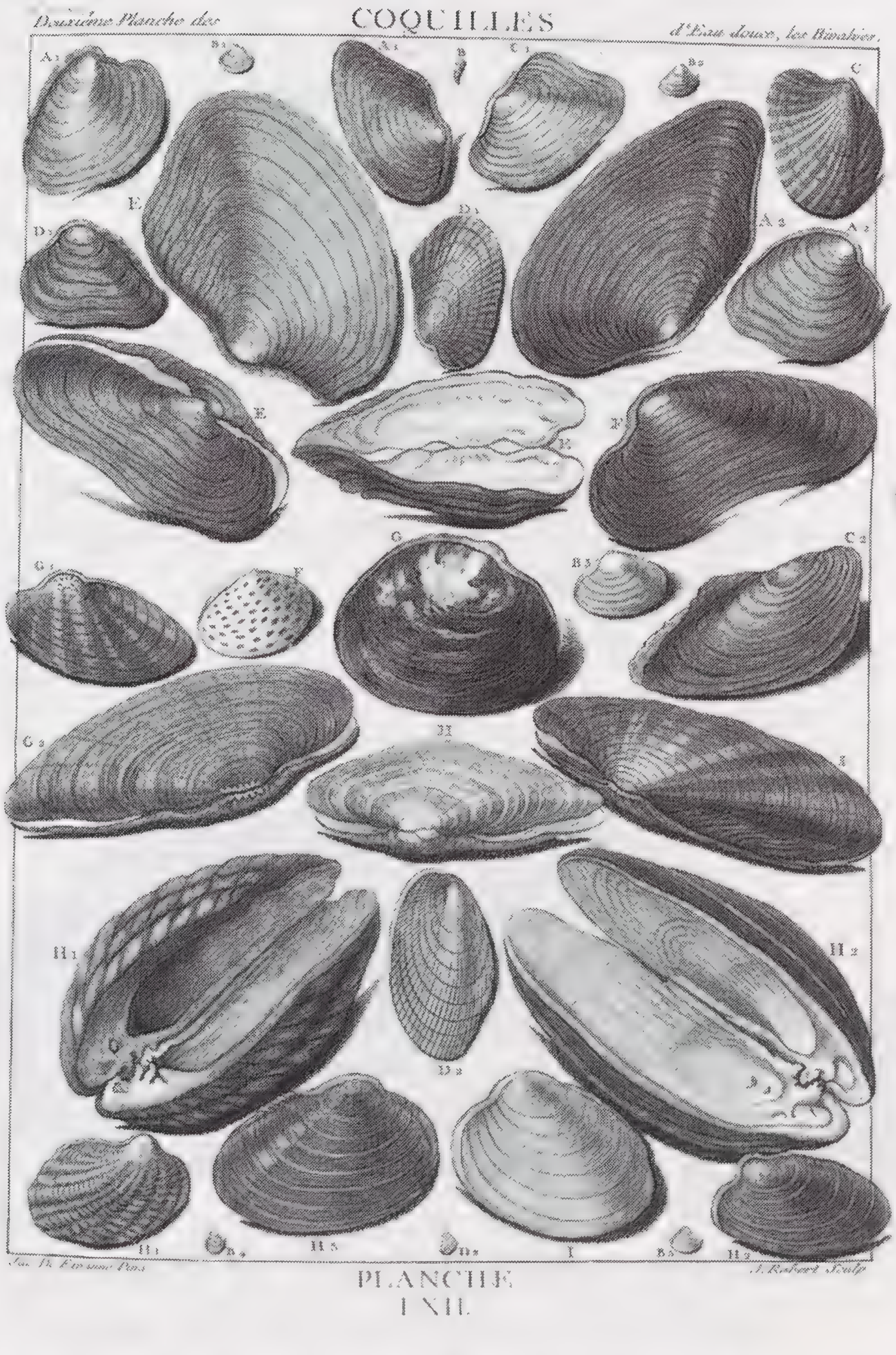

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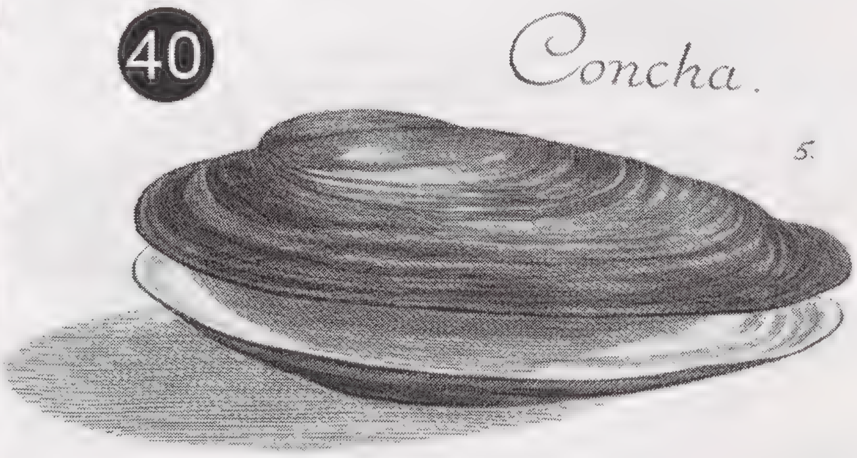

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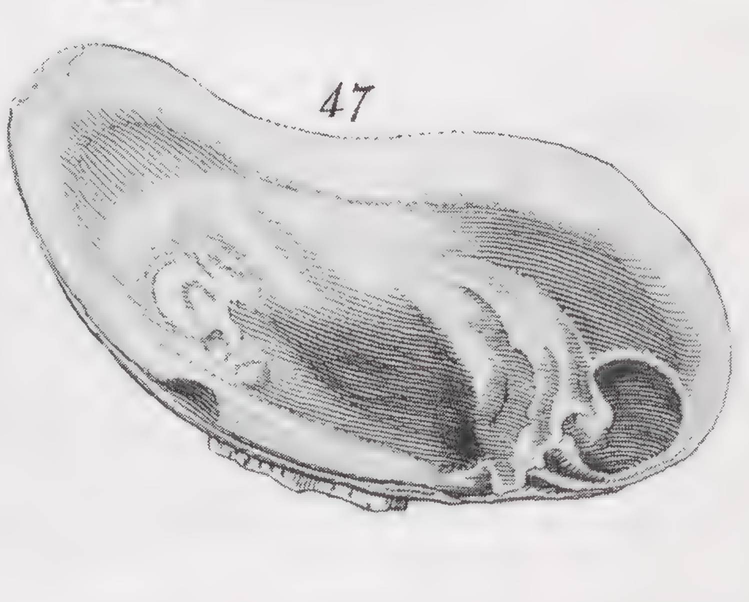
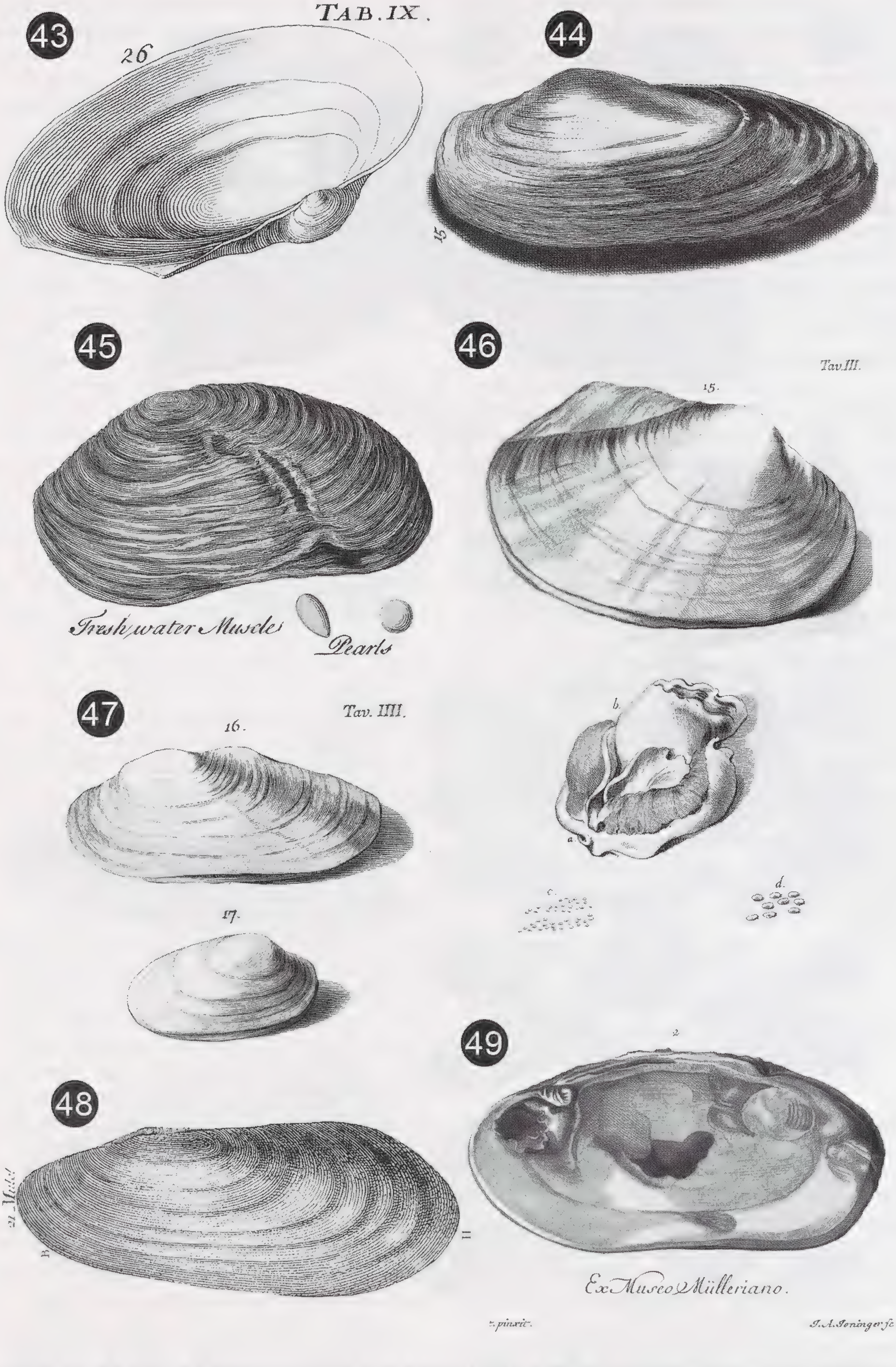


FIGS. 38–42. FIG. 38: *Moules* from D'Argenville (1757); FIG. 39: *Moules* from D'Argenville (1780); FIG. 40: *Moule* from Marsigli (1744); FIG. 41: External anatomy of a freshwater mussel from Swammerdam (1737); FIG. 42: *Diconcha sulcata* from Klein (1753). FIGS. 38, 39, 40, 42: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 41: By permision of Biblioteca Nacional, Madrid, Spain.



FIGS. 43–49. FIG. 43: *Musculus latus* from Klein (1753); FIG. 44: Unnamed figure from Valentijn (1754); FIG. 45: *Freshwater muscle* and two pearls from Pontoppidan (1755); FIG. 46: *Conca de Pittori* from Ginanni (1757); FIG. 47: *Conca de Pittori* from Ginanni (1757); FIG. 48: *Le mutel* from Adanson (1757); FIG. 49: *La moule a perles de l'Elster* from Knorr (1768). FIGS. 43–49: By permission of Museo Nacional de Ciencias Naturales, Madrid, Spain.

A similar argument in favour of anatomical studies on molluscs was also put forward by D'Argenville (1757), who also implored naturalists to take more interest in living molluscs in admiration for the creations of God.

Heide (1683) presented the first known illustrations of the anatomy of naiads (Heard, 1998). Later ones were provided by Lister (1695), Leeuwenhoek (1697), Hermann (1737), Swammerdam (1737), D'Argenville (1742), and Ginanni (1757). Some of these authors also illustrated the eggs, glochidia and the microscopical architecture of the shells. The only pre-Linnean picture of a living naiad was the drawing by Poupart (1731) discussed above, which was copied by Scheuchzer (1735).

Giuseppe Saverio Poli was the first author who figured the gonad, siphons, gill structure, eggs, and glochidia of naiads in a modern way. The elaborate and lavish plates in the three volumes of his *Testacea utriusque Siciliae*, printed in 1791–1827, are among the most beautiful illustrations of the external appearance and anatomy of freshwater mussels. They are not considered here because that book is clearly post-Linnean. The same occurs with the illustrations by both Etienne Louis Geoffroy (1767) and Johann Samuel Schröter (1779). Schröter published *Die Geschichte der Flussconchylien*, the first book entirely devoted to freshwater molluscs. Its first five plates, hand-coloured in some copies, illustrated practically all the European species of naiads as well as other bivalve species of the families Sphaeriidae and Corbiculidae. Missing was *Margaritifera auricularia* (Spengler, 1793), which despite its discovery and naming by the Danish malacologist Lorentz Spengler, continued to be confused with the pearl-producing, much more depicted *M. margaritifera* (Valledor & Araujo, 2006).

ACKNOWLEDGEMENTS

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INDEX

Taxa in **bold** are new; pages in *italic* indicate figures of taxa.

Acanthinula aculeata 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Acodontaster 262
Achatina fulica 136–139, 141, 142
achatinaceum, Paropeas 136–139, 141
Achatinella mustelina 142
Achatinellidae 142
Achatinellinae 142
Acicula parcelineata 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  perpusilla 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  polita 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Acroloxus coloradensis 36
  lacustris 36
Acrorbis petricola 52, 56
Actaeon edentulus 251
Actaeonidae 260
Actaeonina 262
  cingulata 251
Actaeonoidea 261
Acteon 251, 260, 261
  (Actaeonina) chariis 262
  antarcticus 260
  tornatilis 261
Acteonidae 251, 260
Acteonina 262
Acteonoidea 260, 261
Actinostola 262
aculeata, Acanthinula 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
acuta, Physa 52
  Pleurocera 276
acutocarinata, Elimia 265
  Goniobasis 265–267, 271, 272, 273
  Melania 276
acutus, Pleurocera 276
Adelomelon ancilla 262
  beckii 193–196, *194*, *195*
  brasiliana 196
Adontorhina 373, 376, 377
  cyclia 373, 376, 377
  keegani 373, 376, 377
  lynnae 373, 376, 377
  pisum 374–376, 376, 377
  similis 373, 376, 377
  sphaericosa 373, 376, 377
Aegopinella epipedostoma 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  minor 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  nitidula 83, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  pura 85, 101, 103
Aequipecten 71
Africarion 296
agapetus, Potamolithus 50
Agriolimacidae 284
alatus, Potamilus 392
albus, Stenosemus 173
Alcyonium 262
alderi, Euconulus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
alexandrina, Biomphalaria 223
Aligena 373, 376
  pisum 373, 376, 377
Alinda biplicata 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
alliarius, Oxychilus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 135–139, 141, 142
alpestris, Vertigo 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
alter, Musculus 383, *387*
amarula, Thiara 247
americanista, Pomella 50
Amiantis purpurata 175, 176, 180, 181
Amphibalanus eburneus 185, 188, *188*
Amplirhagada 200, 202, 213, 214
  indistincta 202
  solemiana 202
Ampullariidae 50, 58, 59
Ampullarioidea 229
Amusium 71
Anadara 71
  bonplandiana 67, 72
  occidentalis 67, 70
Anaplocamus 276
anatina, Anodonta 383, 384, 388, *391*, 392
anatinum, Drepanotrema 52
anatome, Musculi fluviatilis 383, *394*
ancilla, Adelomelon 262
Anculosa 276
Ancylidae 26, 48, 52, 56, 58
Ancylinae 25, 26
Ancylus 38
  borealis 27, 31
  (Ferrissia) hendersoni 27, 33
  fluviatilis 42
  oregonensis 27
  ovalis 27, 31
  rivularis 31
  walkeri 27, 32

angelicae, Vitrina 358
angulata, Chaetopleura 173
angulatus, Cyrtograpsus 175
angustior, Musculus 383, 388, *389*
  Vertigo 100
Anisancylus obliquus 52
Anisothyris 70, 71
  iquitensis 70, 71
annularis, Gallandia 280, 336
  Oligolimax 283, 292, *294*, *295*, 321, *324*, 343, 360
  Vitrina 322
Anodonta 382, 383, 388, *391*, 392, 396
  anatina 383, 384, 388, *391*, 392
  cygnea 381–384, 392
Anomia ephippium 388
antarctica, Hymedesmia 262
antarcticus, Acteon 260
Antillorbis nordestensis 52
antivertigo, Vertigo 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
apiculata, Chaetopleura 172, 173
Aplustridae 260
arabica, Arabivitrina 282, 291, 292, *294*, *295*, 299, *300*, *301*, 302
  Vitrina 299
Arabivitrina 279–281, 285–288, *287*, 291, 296–299, 301, 302, 323, 328, 331, 342, 346, 349, 350, 356, 358
  arabica 282, 291, 292, *294*, *295*, 299, *300*, *301*, 302
  brevispira 351
  jansseni 282, 291, 292, *294*, *295*, 299, 301, 302
arbustorum, Arianta 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
arctata, Polymesoda 66
argentina, Neactaeonina 251, *253*, 254, *254*, *256–258*, 260–262
Argna bielzi 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Arianta arbustorum 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Arion intermedius 137, 138, 141
Ariophantidae 280
asellus, Leptochiton 167
Asolene platae 50
  pulchella 50
  spixii 50
aspera, Columella 100
aspersum, Cornu 142
aspii, Physa 52, 56
atlantica, Plutonia 283, 284, 286, *294*, *295*, 296, 298, 328, *329*, *330*, 331, 334
  Viquesnelia 328, 331
augustae, Australocosmia 199, 201, *202*, *203*, 204, *205–207*, 206, 208, 210, 213, *213*, *214*
aurantia, Chilina 51, 56, 58
aurata, Plaxiphora 167, 168, 170, *170–172*, 171, 173
auricularia, Margaritifera 399
australis, Heleobia 48
Australocosmica 199, 201, 202, *202*, *203*, 211, 213–215, *214*
  augustae 199, 201, *202*, *203*, 204, *205–207*, 206, 208, 210, 213, *213*, *214*
  sanctumpatriciusae 199, 201, *202*, *203*, 206, 207, *208–210*, 210, 213, *213*, 214, *214*
  vulcanica 199, 201, *202*, *203*, 208, 209, 211, *211–214*
Axinulus 373, 376, 377
Aylacostoma 56, 58, 230, 247
  chloroticum 50, 58
  guaraniticum 50
  (Hemisinus) lineolatum 231
  lineolata 234
  stigmaticum 50
Azara labiata 69
Azorivitrina 279–281, 291, 293, 298, 302, 306, 331, 334
  brevispira 282, 292, *294*, *295*, 302, 303, 306, *307*, 349, 351
  brumalis 302, 306, 331
  finitima 282, 288, 292, *294*, *295*, 297, 298, 303, *305*, 306, 331
  laxata 282, 292, *294*, *295*, 303, *304*, 331

baccettii, Vitrinobrachium 283, 291, 292, *294*, *295*, 346, 349
bakowskii, Trichia 85
  Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Balanus improvisus 185
Balcanovitrina 339
  dojtshini 339
Balea fallax 87, 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
  stabilis 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
banatica, Drobacia 85
banaticum, Drobacia 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
bartschi, Ferrissia 27, 32
Basistoma 230
beckii, Adelomelon 193–196, *194*, *195*
bicolor, Vitrina 344
bidentata, Clausilia 83, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
bielzi, Argna 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Mastus 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127

Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Biomphalaria alexandrina 223
  glabrata 161
  intermedia 52
  obstructa 161
  occidentalis 52
  oligoza 52
  orbignyi 52
  peregrina 52
  pfeifferi 161–165, *162*
  straminea 52
  tenagophila 52, 161
biplicata, Alinda 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
blanci, Dreissena 190
  Phenacolimax 283, *294*, *295*, 325
blauneri, Insulivitrina 288, 297, 312, 315, *316*
bonelli, Semilimacella 283–285, 291–293, *294*, *295*, 298, 339, 340, *341*, 343, 360
  Vitrina 323, 339
bonplandiana, Anadara 67, 72
borealis, Ancylus *27*, 31
Brachydontes rodriguezii 176
brackebuschii, Plagiodontes 21
Bradybaena fruticum 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  similaris 136, 138
brasiliana, Adelomelon 196
breve, Helicolimax 346
  Vitrinobrachium 283, 292, *294*, *295*, 299, 346, *347*, *348*, 349
brevipes, Daudebardia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
brevispira, Arabivitrina 351
  Azorivitrina 282, 292, *294*, *295*, 302, 303, 306, *307*, 349, 351
brumalis, Azorivitrina 302, 306, 331
Buccinanops 175, 180
  cochlidium 180
  globulosus 175, *176*, 176, 178, *178*, *179*, 180, 181
  moniliferus 180
buccinoides, Hemisinus 229–231, *233*, 234, *235*, 238, 242
  Hemisinus (Basistoma) 234
Buccinum cyaneum 196
  undatum 196
budapestensis, Tandonia 282, 284, 286, 287, 291
bugensis, Dreissena 185
  Dreissena rostriformis 185, 187, 189, 190
Bulgarica cana 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Bulimus daedaleus major 17, 19, 20
  (Odontostomus) daedaleus major 19
  (Odontostomus) daedaleus var. *major* 17
  (Plagiodontes) daedaleus var. *salinicola* 19
  (Plagiodontes) daedaleus var. *strobelii* 19
Bullina 260
Bullinidae 260
buschii, Potamolithus 50

Calidivitrina 280, 281, *287*, 287, 288, 293, 297, 298, 302, 323, 328, 331, 342, 346, 349–351, 356, 358
  cf. *oleosa* 283, 286, 293, *294*, *295*, 350, *351*
californica, Gundlachia *27*, 32
Callochiton 167
  dentatus 173
callosus, Potamolithus 50
calophana, Carpathica 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Calyptraea pileolus 262
Camaenidae 199–201, 213, 214
cana, Bulgarica 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
canaliculata, Melania 276
  Pomacea 50, 161
canariensis, Insulivitrina 312, 315
Canarivitrina 279–281, 285, *287*, 288, 293, 296–298, 306, 309, 312, 315, 331
  dianae 282, 292, *294*, *295*
  taburientensis 282, 292, *294*, *295*, 306, *308*, *310*
Candaharia 296
carinifera, Goniobasis 265, 268, 271, *272*, *273*
  Melania 271
carinthiacus, Semilimax 283, 292, *294*, *295*, 299, 344, *345*, 346
carniolica, Semilimacella 343
caroliniana, Cyclas 63
  Polymesoda 66
Carpathica 296
  calophana 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Carychium minimum 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  tridentatum 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
caspia, Dreissena 189, 190
castellanosae, Heleobia 50, 56
catenaria, Goniobasis 266
catharinae, Potamolithus 50
Causa holosericum 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
cellarius, Oxychilus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 282, 284–288, 292, *294*, *295*
Cepaea hortensis 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  nemoralis 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  vindobonensis 100

cephalonica, Semilimacella 283, 291, 292, *294*, *295*, 340, *342*, 343, 360
Ceriphasia 276
  sulcata 276
Cerithioidea 229
Chaetopleura 167, 172
  angulata 173
  apiculata 172, 173
  isabellei 167, 168, *168*, *169*, 170, 172, 173
Chaetopleuridae 168
charcoti, Tedania 262
chariis, Acteon (Actaeonina) 262
Chilina aurantia 51, 56, 58
  dombeiana 51
  fluminea 51
  fulgurata 51
  gallardoi 51
  gibbosa 51, 58
  guaraniana 51, 56
  iguazuensis 48, 51, 56
  megastoma 51, 56
  mendozana 51
  neuquenensis 51, 56, 58
  parchappii 51
  patagonica 51
  perrieri 51, 56
  portillensis 51, 56
  rushii 51
  strebeli 51, 56, 58
Chilinidae 48, 51, 56, 58
Chilostoma faustinum 87, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Chionopsis 71
Chiton 173
  nigrovirescens 167
Chitonidae 173
Chlamydea 344
chloroticum, Aylacostoma 50, 58
Chondrina clienta 100
christinae, Guerrina 282, 292, *294*, *295*, 312
cimex, Drepanotrema 52
cinerea, Cyanoplax 173
cingulata, Actaeonina 251
  Neactaeonina 251, 252, *259*, 260
Clausilia bidentata 83, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  cruciata 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  dubia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  parvula 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  pumila 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
clavaeformis, Elimia 266
  Goniobasis 265–267, 271, *272*, *273*
clessiniana, Ferrissia (Pettancylus) cf. 42
clienta, Chondrina 100
Cobresia 356
  vitrea 356
Cochlicopa lubrica 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  lubricella 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  nitens 83, 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
cochlidium, Buccinanops 180
Cochliopidae 50, 58
Cochlodina costata 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  laminata 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  orthostoma 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
cojitamboensis, Corbicula (Cyanocyclas) 71
coloradensis, Acroloxus *36*
Columella aspera 100
  edentula 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
columella, Lymnaea 51
Conca de Pittori 384, 396, *398*
concentricus, Uncancylus 52
Concha longa 383, 388, *389*
  fluviatiles margaritifera 384, *395*
  longa pictorum dicta 383, *389*, *390*
  longa zoographi 383
  margaritifera *386*, 388
  pictorum 388
concha, Mytuli 396
Conchae longae 383, *389*
Conchocele 377
concordianus, Potamolithus 50, 56
conexa, Heleobia 48
conicus, Potamolithus 51
conoidea, Vitrina 321, 323
contracta, Vitrea 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
Corbicula 66
  (Corbiculella) tenuis 66
  (Cyanocyclas) cojitamboensis 71
  fluminea 367–370, *369*
  tenuis 66
Corbiculella 66
Corbiculidae 71, 399
Corbula 69, 70
  (Caryocorbula) 71
  mactroides 67, 69
  mactroides var. *paranensis* 69
Corbulidae 70
Cornu aspersum 142
costata, Cochlodina 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128

  Vallonia 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
costatus, Plagiodontes daedaleus 20
Crassostrea 71
  patagonica 71, 72
matericulatus, Maxacteon 261
Crenilabrum 261
  exilis 261
Cristaria plicata 382, 392
cruciata, Clausilia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Cryptochiton 173
  stelleri 173
Cryptozona 280, 290
crystallina, Vitrea 85, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 282, 284, 286, 287, 291, 292, *294*, *295*
Cubaedomus 229, 230
cubensis, Veronicella 136, 137, 139, 141, 142
cumingii, Hyriopsis 382
curtum, Pleurocera 267, 273
cuticula, Guerrina 282, 292, *294*, *295*, 309, *311*, 312
  Helix 309
cyaneum, Buccinum 196
Cyanoplax 167, 173
  cinerea 173
Cyclas caroliniana 63
cyclia, Adontorhina 373, 376, 377
Cyclodontina (Plagiodontes) daedalea major 19
  (Plagiodontes) daedalea minor 19
cygnea, Anodonta 381–384, 392
cylindrica, Truncatellina 100
Cyrena salobris 65
Cyrenidae 63, 66
Cyrtograpsus angulatus 175

daedalens, Plagiodontes 20
daedaleus, Bulimus (Odontostomus) var. *major* 17
  Bulimus (Plagiodontes) var. *salinicola* 19
  Bulimus (Plagiodontes) var. *strobelii* 19
  Odontostomus (Plagiodontes) var. *salinicola* 19
  Plagiodontes 1, 3, 5, *5*, 19–24
  Plagiodontes daedaleus 3–9, *5*, *7*, *8*, *10–12*, 11, 13, 14, *14*, *15*, 17, *18*, 19, 20
Daudebardia 296
  brevipes 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  rufa 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
De Margaritis 383
dealdalea, Plagiodontes 20
dentata, Helix 17
dentatus, Callochiton 173
  Plagiodontes 21
depressissimum, Drepanotrema 52
depressus, Oxychilus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
derasa, Tridacna 157
Deroceras 279, 290
  laeve 136–139, 141, 142, 282, 284, 286, 287, 291
deubeli, Oxychilus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
dianae, Canarivitrina 282, 292, *294*, *295*
diaphana, Eucobresia 85, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, 286, *294*, *295*, 296, 351, 352, *353*, *354*, 356
  Lymnaea 51
  Vitrea 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Vitrina 326, 346, 352
dibothrion, Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
dicta, Concha longa pictorum 383, *389*, *390*
dinochilus, Potamolithus 51
Diplodon cimex 52
  depressissimum 52
  fraus 66
  heloicum 52
  kermatoides 52
  lucidum 52
Diconcha sulcata 384, *397*
Discus perspectivus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  rotundatus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  ruderatus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
doellojuradoi, Erodona 61, 63, 67, *68*, 71
doeringi, Potamolithus 51
doeringy, Potamolithus 56
dojtshini, Balcanovitrina 339
doliolum, Orcula 100
  Sphyradium 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
dombeiana, Chilina 51
draparnaudi, Oxychilus 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Dreissena blanci 190
  bugensis 185
  caspia 189, 190
  elata 189, 190
  polymorpha 185, 187, 189, 190
  presbensis 190
  rostriformis 189
  rostriformis bugensis 185, 187, 189, 190
Dreissenidae 186, 189
Drepanotrema anatinum 52
Drobacia banatica 85
  banaticum 102, 105, 108, 111, 114, 117, 120, 123, 126, 129

dubia, Clausilia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
dufresnei, Zidona 196
dulcis, Musculis aquae 383, *387*, 388
  Musculus aquae *387*
duryi, Helisoma 161

eburneus, Amphibalanus 185, 188, *188*
Eceo la concha margaritifera 383, 385
eceroensis, Insulivitrina 282, *294*, *295*, 312, 315
edentula, Columella 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Neactaeonina 251, 252, *259*, 260
edentulus, Actaeon 251
elata, Dreissena 189, 190
  Vestia 85, 87, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
elegans, Oxyloma 100
Elimia 265, 266, 273, 276
  acutocarinata 265
  clavaeformis 266
  livescens 265, 276
elongata, Felipponea 50
  Vitrina 343, 344, 346
Ena montana 87, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  obscura 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
ephippium, Anomia 388
epipedostoma, Aegopinella 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Erodonidae 67
Erodona 61–63, 67, 69–73
  doellojuradoi 61, 63, 67, *68*, 71
  mactroides 67, 69–72
Eucobresia 279–281, 285, 286, *287*, 288, 293, 296–299, 317, 328, 342, 346, 349, 352, 355, 356, 358
  diaphana 85, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, 286, *294*, *295*, 296, 351, 352, *353*, *354*, 356
  glacialis 283, *294*, *295*, 352, *355*, 356
  nivalis 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, 286, *294*, *295*, 297, 352, 356, *357*
  pegorarii 356
Euconulus 279, 290
  alderi 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  fulvus 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 282, 284, 286–288, *294*, *295*
Euglandina rosea 135–139, *140*, 141, 142
Euomphalia strigella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Eurycaelon 276
excentricus, Hebetancylus 28, 34, 38
exilis, Crenilabrum 261

Fagotia 231
fallax, Balea 87, 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
faustinum, Chilostoma 87, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Felipponea 59
  elongata 50
  iheringi 50
  neritiniformis 50
Ferrissia 25, 26, *27*, 28, *29*, 34, 35, *37*, 38, 40, 41, 43, 265
  bartschi *27*, 32
  (Ferrissia) 43
  (Ferrissia) rivularis 25
  fragilis 25, 26, *27*, 28, *29*, 31–33, 35, *35*, *36*, 38, *39*, *40*, 40–43, *42*
  (Kincaidilla) fragilis 25
  mcneilli 25, 26, *27*, 28, *29*, 30, 32, 35, 38, *39*, *40*, 41, 42
  michiganensis *27*, 32, 41
  parallela 25, 26, 28, *29*, 31, 35, 38, *39*, 40, 41, 43
  (Pettancylus) 38, 42, 43
  (Pettancylus) cf. *clessiniana* 42
  rivularis 25, 26, *27*, 28, *29*, 30, 31, 35, *36*, 38, *39*, *40*, 40–43
  sharpi 25, 28, 34, 38, *39*, 41–43
  walkeri 25, 26, *27*, 28, *29*, 30, 32, 38, *39*, 41, 42
Fijidoma 247
filograna, Ruthenica 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
finitima, Azorivitrina 282, 288, 292, *294*, *295*, 297, 298, 303, *305*, 306, 331
floridana, Polymesoda 67
fluminea, Chilina 51
  Corbicula 367–370, *369*
Fluviatilen, Musculus 384
fluviatiles, Musculus 383
fluviatilis, Ancylus 42
  Musculis *395*
  Musculus 384
Fluvio Tamesi 383
formosana, Oncomelania 161
fragilis, Ferrissia 25, 26, *27*, 28, *29*, 31–33, 35, *35*, *36*, 38, *39*, *40*, 40–43, *42*
  Ferrissia (Kincaidilla) 25
  Neactaeonina 251, 252, *259*, 260
fraus, Diplodon 66
fruticum, Bradybaena 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
fulgurata, Chilina 51
fulica, Achatina 136–139, 141, 142
fulvus, Euconulus 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 282, 284, 286–288, *294*, *295*
fuscus, Laevapex 26, *37*, 38
Fusitriton magellanicus 262

Galba truncatula 48, 51
Gallandia 280, 321, 323, 336
  annularis 280, 336
  olympica 280, 321, 323, 336
  paulucciae 336
gallardoi, Chilina 51
gibbosa, Chilina 51, 58
gigas, Tridacna 155, *156*, *157*, 157, 158
glaber, Oxychilus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
glabrata, Biomphalaria 161
glacialis, Eucobresia 283, *294*, *295*, 352, *355*, 356
Glacidorbidae 51, 58
globulosus, Buccinanops 175, *176*, 176, 178, *178*, *179*, 180, 181
Glochidium parasiticum 392
gomerensis, Insulivitrina 312, 315
Gondwanorbis magallanicus 51, 56, 58
Goniobasis 265, 266, 273, 276
  acutocarinata 265–267, 271, *272*, 273
  carinifera 265, 268, 271, *272*, 273
  catenaria 266
  clavaeformis 265–267, 271, *272*, 273
  livescens 265, 266
  osculata 276
  proxima 266
  simplex 265–268, *269*, 271, *272*
Gorgonocephalus 262
granifera, Tarebia 247
granulata, Neohelice 175
guangxiensis, Oncomelania hupensis 147, 152
guaraniana, Chilina 51, 56
guaranitica, Heleobia 50
guaraniticum, Aylacostoma 50
Guerrina 279–281, 285, *287*, 288, 293, 296–298, 309, 312, 315, 351
  christinae 282, 292, *294*, *295*, 312
  cuticula 282, 292, *294*, *295*, 309, *311*, 312
gulo, Vestia 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Gundlachia 38
  californica *27*, 32
  ticaga 52, 56
Gymnarion 296
Gyrotoma 276

Haemisinus 231
Haliclona kerguelensis 262
Haliotis rubra 180, 181, 196
hammonis, Nesovitrea 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
hatcheri, Heleobia 50
Hebetancylus excentricus 28, 34, 38
  moricandi 52
Heleobia australis 48
  castellanosae 50, 56
  conexa 48
  guaranitica 50
  hatcheri 50
  isabelleana 50, 56
  kuesteri 50, 56
  montana 50
  occidentalis 50, 56
  parchappii 50
  peiranoi 50, 56
  piscium 50
  rionegrina 50, 56
  sublineata 50, 58
  tucumana 50
  vianai 50, 56
Helicarionoidea 280
Helicigona lapicida 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Helicodonta obvoluta 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Helicoidea 199
Helicolimax 356
  breve 346
  lamarckii 312
  major 325, 326
Helisoma duryi 161
Helix cuticula 309
  dentata 17
  lutescens 100
  pellucida 356
  pomatia 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  semilimax 343, 344
heloicum, Drepanotrema 52
Hemisinus 229–231, *232*, *233*, 234, *235*, 242, 245–247
  (Basistoma) buccinoides 234
  (Basistoma) lineolatus 231
  buccinoides 229–231, 233, 234, *235*, 238, 242
  (Hemisinus) lineolatus 231
  lineolata 231, 234
  lineolatus 229–231, *232–235*, 234, 236, *237*, 238, *239–245*, 242, 245–247
  lineolatus var. *buccinoides* 234
hendersoni, Ancylus (Ferrissia) *27*, 33
Hessemilimax 280, 281, 288, 297–299, 336, 339, 342, 346
  kotulae 283, 292, 293, *294*, *295*, 296, 299, 336, *337*, *338*, 339, 360
heterostropha, Physa 161
Hiatella meridionalis 262
hidalgo, Potamolithus 51
hispidus, Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
holosericum, Causa 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
hortensis, Cepaea 102, 105, 108, 111, 114, 117, 120, 123, 126, 129

hupensis, Oncomelania 147–149, 152, 161, 217, 218, *219*, 220–222
  Oncomelania hupensis 147, 148, *152*, 152
Hyalina 356
Hymedesmia antarctica 262
Hyriopsis cumingii 382

iguazuensis, Chilina 48, 51, 56
iheringi, Felipponea 50
improvisus, Balanus 185
incarnata, Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
indistincta, Amplirhagada 202
inexpectata, Neactaeonina 251, 252, *259*, 260
inopinatus, Oxychilus 100
insularum, Pomacea 50
Insulivitrina 279–281, 285, *287*, 288, 293, 297, 298, 302, 309, 312, 315, 336, 356
  blauneri 288, 297, 312, 315, *316*
  canariensis 312, 315
  eceroensis 282, *294*, *295*, 312, 315
  gomerensis 312, 315
  lamarckii 282, 288, *294*, *295*, 297, 298, 312, *313*, *314*, 315
  oromii 312, 315
  parryi 315
  teneriffae 315
intermedia, Biomphalaria 52
intermedius, Arion 137, 138, 141
Io 276
Iophon proximum 262
iquitensis, Anisothyris 70, 71
isabelleana, Heleobia 50, 56
  Potamolithus 51
isabellei, Chaetopleura 167, 168, *168*, *169*, 170, 172, 173
Isodictya verrucosa 262
Isognomostoma isognomostoma 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
isognomostoma, Isognomostoma 102, 105, 108, 111, 114, 117, 120, 123, 126, 129

jansseni, Arabivitrina 282, 291, 292, *294*, *295*, 299, 301, 302
Japonacteon 261
  pusillus 261
japonicum, Schistosoma 147, 152, 217
Juga 276

kamehamehae, Plaxiphora 173
Katharina tunicata 173
keegani, Adontorhina 373, 376, 377
kerguelensis, Haliclona 262
kermatoides, Drepanotrema 52
Kimberleydiscus 200
Kimboraga 202
Kincaidilla 25, 43
kotulae, Hessemilimax 283, 292, 293, *294*, *295*, 296, 299, 336, *337*, *338*, 339, 360
  Semilimax 87, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Vitrina 336
kuesteri, Heleobia 50, 56

La moule a perles de l'Elster 384, 396, *398*
labiata, Azara 69
Labidiaster 262
Laciniaria plicata 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
lacustris, Acroloxus *36*
Laevapex 26, 48
  fuscus 34, *37*, 38
laeve, Deroceras 136–139, 141, 142, 282, 284, 286, 287, 291
lamarckii, Helicolimax 312
  Insulivitrina 282, 288, *294*, *295*, 297, 298, 312, *313*, *314*, 315
laminata, Cochlodina 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Lampadia lederi 334
lapicida, Helicigona 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
lapidum, Potamolithus 51
laqueata, Melania 276
latestriata, Macrogastra 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
latus, Musculus 383, 384, 388, *389*, 396, *398*
Lavigeria 246
laxata, Azorivitrina 282, 292, *294*, *295*, 303, *304*, 331
  Plutonia 288
  Vitrina 302
Le Mutel 384, 396, *398*
lederi, Lampadia 334
  Oligolimax 323
  Trochovitrina 280, 283, 284, 286, *294*, *295*, 334, *335*, 336
Lepidochitona 167, 173
Lepidochitonidae 167
leptochela, Phorbas 262
Leptochiton asellus 167
Leptoxis 276
leucophaeata, Mytilopsis 185–190, *186*, *188*
Libania 296
Libinia spinosa 181
Limacoidea 279
Limatula pygmaea 262
Limax maximus 142
lineata, Melanopsis 231
  Tonicella 173
lineatus, Mytilaster 190
lineolata, Aylacostoma 234

  Hemisinus 231, 234
  Melania 231, 234
  Melania (Hemisinus) 231
  Melanopsis 231
  Semisinus 231
lineolatum, Aylacostoma (Hemisinus) 231
lineolatus, Hemisinus (Basistoma) 231
  Hemisinus 229–231, *232–235*, 234, 236, *237*, 238, *239–245*, 242, 245–247
  Hemisinus (Hemisinus) 231
  Hemisinus var. *buccinoides* 234
  Melanopsis 231
  Semisinus 231
  Strombus 231, 234
Lithasia 265, 276
Lithoglyphidae 50, 56, 58, 59
Littorinidae 244
livescens, Elimia 265, 276
  Goniobasis 265, 266
longa, Concha 383, *389*
longae, Conchae 383, 388, *389*
Longiverena 230
loosi, Physa 52, 56
lubomirskii, Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
lubrica, Cochlicopa 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
lubricella, Cochlicopa 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
lucidum, Drepanotrema 52
lutescens, Helix 100
lynnae, Adontorhina 373, 376, 377
Lymnaea 265
  columella 51
  diaphana 51
  peregra 161
  pictonica 52, 58
  plicata 52, 58
  viatrix 52
Lymnaeidae 48, 51
Lyonsiidae 70

Macrogastra latestriata 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  plicatula 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  tumida 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  ventricosa 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Macrolimen 276
Mactra 71
mactroides, Corbula 67, 69
  Corbula var. *paranensis* 69
  Erodona 67, 69–72
Madeirovitrina 280, 281, 285, *287*, 288, 293, 297, 298, 309, 315, 317, 318, 334, 351
  marcida 282, 292, *294*, *295*, 298, 315, 317, 318, *319*, *320*
  media 349, 351
  nitida 282, 292, *294*, *295*, 298, 315, 317, *318*
magallanicus, Gondwanorbis 51, 56, 58
magellanica, Odontocymbiola 181, 262
magellanicus, Fusitriton 262
major, Bulimus daedaleus 17, 19, 20
  Bulimus (Odontostomus) daedaleus 19
  Cyclodontina (Plagiodontes) daedalea 19
  Helicolimax 325, 326
  Phenacolimax 283, *294*, *295*, 298, 299, 325, *326*, *327*, 328
  Plagiodontes 20
  Plagiodontes daedaleus 1, 19, 20
mansoni, Schistosoma 161
marcida, Madeirovitrina 282, 292, *294*, *295*, 315, 317, 318, *319*, *320*
Margaritifera 396
  auricularia 399
  margaritifera 381–385, *386*, 388, *390*, 392, 396, 399
margaritifera, Concha *386*, 388
  Concha fluviatiles 384, *395*
  Eceo la concha 383, 385
  Margaritifera 381–385, *386*, 388, *390*, 392, 396, 399
  Pinctada 382, 388
Margaritiferidae 381
Marisa planogyra 50
marmorata, Stenophysa 52
massa, Tedania 262
Mastus bielzi 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Maxacteon 261
  cratericulatus 261
maximus, Limax 142
  Musculus 383
  Musculus tenuis 388
mcneilli, Ferrissia 25, 26, *27*, 28, *29*, 30, 32, 35, 38, *39*, *40*, 41, 42
media, Madeirovitrina 349, 351
megastoma, Chilina 51, 56
  Pomella 50
Meghimatium striatum 142
Melania 231
  acutocarinata 276
  canaliculata 276
  carinifera 271
  (Hemisinus) lineolata 231
  laqueata 276
  lineolata 231, 234

  olivula 276
  plena 276
  showalterii 276
  undulata 276
Melanoides 247
  tuberculata 247
  tuberculatus 50
Melanopsis lineata 231
  lineolata 231
  lineolatus 231
Melasma 276
Mendicula 377
mendozana, Chilina 51
meridionalis, Hiatella 262
michiganensis, Ferrissia *27*, 32, 41
microthauma, Potamolithus 51
minimum, Carychium 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
minor, Aegopinella 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  Cyclodontina (Plagiodontes) daedalea 19
  Musculus 383
  Musculus tenuis 388
  Plagiodontes daedaleus 19, 20
modus, Scottorum in fluviis piscandi 383, 388
moniliferus, Buccinanops 180
montana, Ena 87, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Heleobia 50
Mopalia 173
  muscosa 173
Mopaliidae 167, 170, 173
moricandi, Hebetancylus 52
mosorensis, Troglaegopis 282, 284, 286–288, *289*, 291, 292, *294*, *295*
Moule 384, *394*, *395*, *397*
mucosa, Tedania 262
Mudalia 276
multidentatus, Plagiodontes daedaleus 20
muravchiki, Polymesoda 61–63, *64*, 65–67
muscosa, Mopalia 173
  Vitrina 321, 323
Musculi fluviatilis anatome 383, *394*
Musculis aquae dulcis 383, *387*, 388
  aquae dulcis Rondeletius 383, 388
  fluviatilis 395
Musculus 383, *387*, 388, 392
  alter 383, *387*
  angustior 383, 388, *389*
  aquae dulcis 387
  Fluviatilen 384
  fluviatiles 383
  fluviatilis 384
  latus 383, 384, 388, *389*, 396, *398*
  maximus 383
  minor 383
  niger 383, 388
  tenuis 383
  tenuis maximus 388
  tenuis minor 388
musignani, Oligolimax 283, 292, *294*, *295*, 321, *322*, *323*, 343, 360
mustelina, Achatinella 142
Mutela 396
Mutelidae 384
Myoidea 67
Mytilaster lineatus 190
Mytilopsis 189, 190
  leucophaeata 185–190, *186*, *188*
Mytuli 384
  concha 396

Nassariidae 175
Neactaeonina 251, 252, *259*, 260–262
  argentina 251, *253*, 254, *254*, *256–258*, 260–262
  cingulata 251, 252, *259*, 260
  edentula 251, 252, *259*, 260
  fragilis 251, 252, *259*, 260
  inexpectata 251, 252, *259*, 260
nemoralis, Cepaea 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Neocorbicula 65, 66
  stelzneri 67
Neohelice granulata 175
neritiniformis, Felipponea 50
Nesovitrea hammonis 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  petronella 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
neuquenensis, Chilina 51, 56, 58
niger, Musculus 383, 388
nigrovirescens, Chiton 167
nitens, Cochlicopa 83, 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
nitida, Madeirovitrina 282, 292, *294*, *295*, 315, 317, *318*
  Vitrina 315
nitidula, Aegopinella 83, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
nitidus, Zonitoides 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Nitocris 276
nivalis, Eucobresia 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, 286, *294*, *295*, 297, 352, 356, *357*
nordestensis, Antillorbis 52
notabilis, Polymesoda 67

obliquirugosa, Xanthomelon 200, *213*
obliquus, Anisancylus 52

  Pachydon 70
oblonga, Succinea 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
obscura, Ena 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
obstructa, Biomphalaria 161
obvoluta, Helicodonta 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
occidentalis, Anadara 67, 70
  Biomphalaria 52
  Heleobia 50, 56
Odontaster 262
Odontocymbiola magellanica 181, 262
Odontostominae 1, 17
Odontostomus (Plagiodontes) daedaleus var. *salinicola* 19
oleosa, Calidivitrina cf. 283, 286, 293, *294*, *295*, 350, *351*
  Vitrina 350
Oligolimacini 281
Oligolimax 279–281, 285–287, *287*, 288, 293, 296–299, 302, 315, 321–324, 328, 331, 334, 336, 342, 346, 349, 350, 356, 358
  annularis 283, 292, *294*, *295*, 321, *324*, 343, 360
  lederi 323
  musignani 283, 292, *294*, *295*, 321, *322*, *323*, 343, 360
  olympicus 321
  pollonerianus 323
  sturanyi 323
oligoza, Biomphalaria 52
olivula, Melania 276
olympica, Gallandia 280, 321, 323, 336
olympicus, Oligolimax 321
Oncomelania 217
  formosana 161
  hupensis 147–149, 152, 161, 217, 218, *219*, 220–222
  hupensis guangxiensis 147, 152
  hupensis hupensis 147, 148, *152*, 152
  hupensis robertsoni 147, 148, 152
  hupensis tangi 147, 152
Ophiacanta 262
Ophiomorpha 63
orbignyi, Biomphalaria 52
  Potamolithus 51
Orcula doliolum 100
oregonensis, Ancylus 27
oromii, Insulivitrina 312, 315
Orthalicidae 1, 17
orthostoma, Cochlodina 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
osculata, Goniobasis 276
Ostomya 70
  papyria 70
Ovalipes trimaculatus 181
ovalis, Ancylus 27, 31
Oxychilus 279, 286, *287*, 290
  alliarius 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 135–139, 141, 142
  cellarius 101, 104, 107, 110, 113, 116, 119, 122, 125, 128, 282, 284–288, 292, *294*, *295*
  depressus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  deubeli 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  draparnaudi 85, 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  glaber 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
  inopinatus 100
Oxyloma elegans 100
Oxytrema 276

Pachydon 70, 71
  obliquus 70
Pachymelania 247
Pagana 356
pagodula, Pagodulina 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Pagodulina pagodula 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Paludomidae 229, 245, 246
papyria, Ostomya 70
parallela, Ferrissia 25, 26, 28, *29*, 31, 35, 38, *39*, 40, 41, 43
paranensis, Potamolithus 51
parasiticum, Glochidium 392
Parathyasira 377
parcelineata, Acicula 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
parchappii, Chilina 51
  Heleobia 50
Parmacella 296
Paropeas achatinaceum 136–139, 141
parryi, Insulivitrina 315
Partulidae 142
parvula, Clausilia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
parvus, Plagiodontes multiplicatus 21
patagonica, Chilina 51
  Crassostrea 71, 72
  Zygochlamys 252, 260, 262
patagonicus, Plagiodontes 21
paulucciae, Gallandia 336
  Vitrina 321–323
  Vitrina (Oligolimax) 321
pectinata, Siphonaria 35
Pectunculus 383
  subviridis 383, 392

  triquetrus 383
pegorarii, Eucobresia 356
peiranoi, Heleobia 50, 56
pelagica, Plutonia 288
pellucida, Helix 356
  Vitrina 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283–285, 288, 291–293, *294*, *295*, 326, 343, 351, 356, 358, *359*, 360
peregra, Lymnaea 161
peregrina, Biomphalaria 52
Perforatella bidentata 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  dibothrion 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  incarnata 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  rubiginosa 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  umbrosa 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  vicina 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
peristomatus, Potamolithus 51
perpusilla, Acicula 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
perrieri, Chilina 51, 56
perspectivus, Discus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
petitianus, Potamolithus 51
petricola, Acrorbis 52, 56
petronella, Nesovitrea 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Pettancylus 26, 43
pfeifferi, Biomphalaria 161–165, *162*
Phenacolimax 279–281, 285, 286, *287*, 288, 293, 297–299, 302, 312, 315, 317, 322, 323, 325, 326, 328, 331, 342, 346, 349, 356, 358
  blanci 283, *294*, *295*, 325
  major 283, *294*, *295*, 298, 299, 325, *326*, *327*, 328
philipianus, Potamolithus 51
Philonesia 137, 138, 141
Phorbas leptochela 262
Physa acuta 52
  aspii 52, 56
  heterostropha 161
  loosi 52, 56
Physella venustula 52
Physidae 52, 58
pictonica, Lymnaea 52, 58
pictorum, Concha 388
  Unio 382–384, 388, *391*
pileolus, Calyptraea 262
Pinctada margaritifera 382, 388
  radiata 382
Pisciculus testaceus 392
piscium, Heleobia 50
pistillata, Stylophora *157*
pisum, Adontorhina *374–376*, 376, 377
  Aligena 373, 376, 377
Pittori, Conca de 384, 396, *398*
Placunanomia 71
Plagiodontes 1, *4*, *5*, 17, 20, 21
  brackebuschii 21
  daedalens 20
  daedaleus 1, 3, 5, *5*, 19–24
  daedaleus costatus 20
  daedaleus daedaleus 3–9, *5*, *7*, *8*, *10–12*, 11, 13, 14, *14*, *15*, 17, *18*, 19, 20
  daedaleus major 1, 19, 20
  daedaleus minor 19, 20
  daedaleus multidentatus 20
  daedaleus salinicola 1, 19, 20
  daedaleus strobelii 1, 3–9, *5*, *7*, *9*, *10–12*, 11, 13, 14, *14*, *16*, 17, *18*, 19, 20
  dealdalea 20
  dentatus 21
  major 20
  multiplicatus parvus 21
  patagonicus 21
  rocae 21
  strobelii 1, 3, 5, 17, 19–24
  weyenberghii 21
  weyrauchi 21
planogyra, Marisa 50
Planorbidae 25, 52, 56, 58
platae, Asolene 50
Plaxiphora 167, 173
  aurata 167, 168, 170, *170–172*, 171, 173
  kamehamehae 173
plena, Melania 276
Pleurocera 265, 266, 273, 276, 277
  acuta 276
  acutus 276
  curtum 267, 273
  unciale 265–267, 271, *272*, 273
  uncialis 266
  vestita 265, 268, 271, *272*, 273
Pleuroceridae 265, 276, 277
plicata, Cristaria 382, 392
  Laciniaria 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
  Lymnaea 52, 58
plicatula, Macrogastra 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Plutonia 279–281, 285–288, 293, 296–299, 302, 309, 312, 315, 317, 323, 328, 331, 342, 346, 349, 350, 356, 358
  atlantica 283, 284, 286, *294*, *295*, 296, 298, 328, *329*, *330*, 331, 334
  (Canarivitrina) taburientensis 306

  laxata 288
  pelagica 288
Plutoniainae 281
Plutoniidae 331
polita, Acicula 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
polloneriana, Sardovitrina 283, 292, *294*, *295*, *332*, 332, *333*, 343, 360
  Vitrina 331
pollonerianus, Oligolimax 323
Polymesoda 61, 63, 65, 66, 71–73
  arctata 66
  caroliniana 66
  (Egeta) salobris 66
  floridana 67
  muravchiki 61–63, *64*, 65–67
  notabilis 67
  salobris 66
polymorpha, Dreissena 185, 187, 189, 190
Pomacea canaliculata 50, 161
  insularum 50
  scalaris 50
pomatia, Helix 85, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Pomatiopsidae 147
Pomella americanista 50
  megastoma 50
Porania 262
portillensis, Chilina 51, 56
Portlandia 71
Potamilus alatus 392
Potamolithus agapetus 50
  buschii 50
  callosus 50
  catharinae 50
  concordianus 50, 56
  conicus 51
  dinochilus 51
  doeringi 51
  doeringy 56
  hidalgoi 51
  iheringi 51
  lapidum 51
  microthauma 51
  orbignyi 51
  paranensis 51
  peristomatus 51
  petitianus 51
  philipianus 51
  quadratus 51, 56
  rushii 51
  simplex 51
  tricostatus 51, 56
  valchetensis 51, 56
presbensis, Dreissena 190
proxima, Goniobasis 266
proximum, Iophon 262
Pseudocyrena 65
pulchella, Asolene 50
  Vallonia 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
pumila, Clausilia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
Punctum pygmaeum 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Pupa 261
pura, Aegopinella 85, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
purpurata, Amiantis 175, 176, 180, 181
pusilla, Pyramidula 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  Vertigo 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
pusillus, Japonacteon 261
putris, Succinea 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
pygmaea, Limatula 262
pygmaeum, Punctum 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Pyramidula pusilla 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  rupestris 100
pyrenaicus, Semilimax 283, 286, 292, *294*, *295*, 298, 344, 346

quadrates, Potamolithus 51, 56
Quistrachia 202

radiata, Pinctada 382
reitteri, Vitrina 339
Rictaxis 260
rionegrinam, Heleobia 50, 56
rivularis, Ancylus 31
  Ferrissia 25, 26, *27*, 28, *29*, 30, 31, 35, *36*, 38, *39*, *40*, 40–43
  Ferrissia (Ferrissia) 25
Rhodacmea 38
Rhyssoplax tulipa 167
robertsoni, Oncomelania hupensis 147, 148, 152
rocae, Plagiodontes 21
rodriguezii, Brachydontes 176
Rondeletius, Musculis aquae dulcis 383, 388
rosea, Euglandina 135–139, *140*, 141, 142
rostriformis, Dreissena 189
rotundatus, Discus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
rubiginosa, Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
rubra, Haliotis 180, 181, 196
ruderatus, Discus 100, 103, 106, 109, 112, 115, 118, 121, 124, 127

rufa, Daudebardia 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
rugulosa, Vitrina 358
rupestris, Pyramidula 100
rushii, Chilina 51
  Potamolithus 51
Ruthenica filograna 101, 104, 107, 110, 113, 116, 119, 122, 125, 128

salinicola, Plagiodontes daedaleus 1, 19, 20
salobris, Cyrena 65
  Polymesoda 66
  Polymesoda (Egeta) 66
sanctumpatriciusae, Australocosmia 199, 201, *202*, *203*, 206, 207, *208–210*, 210, 213, *213*, *214*, 214
Sardovitrina 279–281, 285–287, *287*, 293, 297–299, 323, 324, 331, 334, 336
  polloneriana 283, 292, *294*, *295*, *332*, 332, *333*, 343, 360
sarsi, Succinea 100
scabra, Thiara 247
Scalarinella (Plagiodontes) daedaleus strobelii 19
scalaris, Pomacea 50
Schistosoma japonicum 147, 152, 217
  mansoni 161
Scottorum in fluviis piscandi modus 383, 388
Semilimacella 279–281, 286–288, *287*, 293, 297–299, 323, 324, 334, 336, 339, 342, 343, 346, 349, 358
  bonelli 283–285, 291–293, *294*, *295*, 298, 339, 340, *341*, 343, 360
  carniolica 343
  cephalonica 283, 291, 292, *294*, *295*, 340, *341*, 343, 360
Semilimacinae 281
Semilimacini 281
Semilimax 280, 281, 286, *287*, 288, 293, 296–299, 336, 339, 342–344, 346, 349, 350, 355, 356
  carinthiacus 283, 292, *294*, *295*, 299, 344, *345*, 346
  kotulae 87, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
  pyrenaicus 283, 286, 292, *294*, *295*, 298, 344, 346
  semilimax 100, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, *285*, 286, 292, *294*, *295*, 298, 299, 336, *343*, 344, 346
semilimax, Helix 343, 344
  Semilimax 100, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283, *285*, 286, 292, *294*, *295*, 298, 299, 336, *343*, 344, 346
Semisinus 230, 231
  lineolata 231
  lineolatus 231
Semisulcospira 265, 273
septentriones, Sypharochiton 173
sharpi, Ferrissia 25, 28, 34, 38, *39*, 41–43
showalterii, Melania 276
similaris, Bradybaena 136, 138
similis, Adontorhina 373, 376, 377
simplex, Goniobasis 265–268, *269*, 271, *272*
  Potamolithus 51
Siphonaria pectinata *35*
solemiana, Amplirhagada 202
sphaericosa, Adontorhina 373, 376, 377
Sphaeriidae 399
Sphyradium doliolum 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
spinosa, Libinia 181
spixii, Asolene 50
stabilis, Balea 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
stelleri, Cryptochiton 173
stelzneri, Neocorbicula 67
Stenophysa marmorata 52
Stenosemus albus 173
Sterechinus 262
stigmaticum, Aylacostoma 50
straminea, Biomphalaria 52
strebeli, Chilina 51, 56, 58
Strephobasis 276
Strepoma 276
striatum, Meghimatium 142
strigella, Euomphalia 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
strobelii, Plagiodontes 1, 3, 5, 17, 19–24
  Plagiodontes daedaleus 1, 3–9, *5*, *7*, *9*, *10–12*, 11, 13, 14, *14*, *16*, 17, *18*, 19, 20
  Scalarinella (Plagiodontes) daedaleus 19
Strombus 231, 234
  lineolatus 231, 234
sturanyi, Oligolimax 323
Stylophora *157*
  pistillata *157*
subcarinata, Vitrina 334
  Vitrina (Trochovitrina) 334
sublineata, Heleobia 50, 58
substriata, Vertigo 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
subviridis, Pectunculus 383, 392
Succinea 137–139, 141
  oblonga 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  putris 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  sarsi 100
  tenella 136–139, 141
sulcata, Ceriphasia 276
  Diconcha 384, *397*
Sypharochiton septentriones 173

taburientensis, Canarivitrina 282, 292, *294*, *295*, 306, *308*, *310*
  Plutonia (Canarivitrina) 306
Tamesi, Fluvio 383
Tandonia 279, 290
  budapestensis 282, 284, 286, 287, 291
tangi, Oncomelania hupensis 147, 152
Tania 231
Tarebia 247
  granifera 247
Targionia 339, 340
Tedania charcoti 262
  massa 262
  mucosa 262
Telescopella 276
tenagophila, Biomphalaria 52, 161
tenella, Succinea 136–139, 141
teneriffae, Insulivitrina 315
tenuis, Corbicula 66
  Corbicula (Corbiculella) 66
  Musculus 383
Testacella 296
testaceus, Pisciculus 392
Thiara 246
  amarula 247
  scabra 247
Thiaridae 50, 56, 58, 229, 230, 242, 245–247
Thouarella 262
Thyasira 377
Thyasiridae 373
ticaga, Gundlachia 52, 56
Tiphobia 246
Tonicella 173
  lineata 173
Tonicia 167
Tornatellides 137, 138, 141
tornatilis, Acteon 261
Torresitrachia 214
Tozzettia 339, 340
transsylvanica, Vitrea 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
Trichia 100
  bakowskii 85
tricostatus, Potamolithus 51, 56
Tridacna 157, 158
  derasa 157
  gigas 155, *156*, *157*, 157, 158
Tridacninae 155
tridentatum, Carychium 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
tridentinum, Vitrinobrachium 349
trimaculatus, Ovalipes 181
triquetrus, Pectunculus 383
Trochovitrina 280, 281, 285, 287, 296–298, 323, 334, 336
  lederi 280, 283, 284, 286, *294*, *295*, 334, *335*, 336
Trochulus 100
  bakowskii 102
  bielzi 102
  hispidus 102
  lubomirskii 102
  unidentatus 102
  villosulus 102
Troglaegopis 279, 280, 288, 290
  mosorensis 282, 284, 286–288, *289*, 291, 292, *294*, *295*
Truncatellina cylindrica 100
truncatula, Galba 48, 51
Trypanostoma 276
tuberculata, Melanoides 247
tuberculatus, Melanoides 50
tucumana, Heleobia 50
tulipa, Rhyssoplax 167
tumida, Macrogastra 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
tumidus, Unio 383, 392, *393*
tunicata, Katharina 173
turgida, Vestia 102, 105, 108, 111, 114, 117, 120, 123, 126, 129

umbrosa, Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Uncancylus 34, 38
  concentricus 52
unciale, Pleurocera 265–267, 271, *272*, 273
uncialis, Pleurocera 266
undatum, Buccinum 196
undulata, Melania 276
unidentatus, Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
Unio 384, 388, 396
  pictorum 382–384, 388, *391*
  tumidus 383, 392, *393*
Unionidae 381, 383, 384

valchetensis, Potamolithus 51, 56
Vallonia costata 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  pulchella 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
velebitica, Vitrina 339
  Vitrina (Semilimacella) 339
ventricosa, Macrogastra 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
venustula, Physella 52
Verena 230
Veronicella cubensis 136, 137, 139, 141, 142
verrucosa, Isodictya 262

Vertigo alpestris 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  angustior 100
  antivertigo 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  pusilla 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
  substriata 100, 103, 106, 109, 112, 115, 118, 121, 124, 127
Vestia 85, 100, 102, 104, 107, 110, 113, 116, 119, 122, 125, 128
  elata 85, 87, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  gulo 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
  turgida 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
vestita, Pleurocera 265, 268, 271, *272*, 273
vianai, Heleobia 50, 56
viatrix, Lymnaea 52
vicina, Perforatella 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
villosulus, Trochulus 102, 105, 108, 111, 114, 117, 120, 123, 126, 129
vindobonensis, Cepaea 100
Vinundu 246
Viquesnelia 328, 331
  atlantica 328, 331
Vitrea 279, 290
  contracta 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
  crystallina 85, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 282, 284, 286, 287, 291, 292, *294*, *295*
  diaphana 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
  transsylvanica 101, 103, 106, 109, 112, 115, 118, 121, 124, 127
vitrea, Cobresia 356
Vitreidae 284
Vitrina 279–281, 286–288, *287*, 293, 296–299, 312, 315, 322–324, 328, 334, 336, 342, 346, 349, 350, 356, 358
  angelicae 358
  annularis 322
  arabica 299
  bicolor 344
  bonelli 323, 339
  conoidea 321, 323
  diaphana 326, 346, 352
  elongata 343, 344, 346
  (Insulivitrina) 302
  kotulae 336
  laxata 302
  musignani 321, 323
  nitida 315
  oleosa 350
  (Oligolimax) paulucciae 321
  paulucciae 321–323
  pellucida 85, 100, 103, 106, 109, 112, 115, 118, 121, 124, 127, 283–285, 288, 291–293, *294*, *295*, 326, 343, 351, 356, 358, *359*, 360
  (Phenacolimax) 302
  polloneriana 331
  reitteri 339
  rugulosa 358
  (Semilimacella) velebitica 339
  subcarinata 334
  (Trochovitrina) subcarinata 334
  velebitica 339
  (Vitrina) 350, 356
Vitrinidae 279, 281, 293
Vitrininae 281
Vitrinizonites 296
Vitrinobrachium 279–281, 284, 286–288, *287*, 291, 293, 296–299, 336, 346, 349
  baccettii 283, 291, 292, *294*, *295*, 346, 349
  breve 283, 292, *294*, *295*, 299, 346, *347*, *348*, 349
  tridentinum 349
Vitrinopugio 344
Vitriplutonia 328
Volutidae 193
vulcanica, Australocosmia 199, 201, *202*, *203*, 208, 209, 211, *211–214*

walkeri, Ancylus *27*, 32
  Ferrissia 25, 26, *27*, 28, *29*, 30, 32, 38, *39*, 41, 42
Watsonula wautieri 43
wautieri, Watsonula 43
weyenberghii, Plagiodontes 21
weyrauchi, Plagiodontes 21

Xanthomelon 215
  obliquirugosa 200, 213

Zidona dufresnei 196
Zonitidae 280
Zonitoidea 280
Zonitoides nitidus 101, 104, 107, 110, 113, 116, 119, 122, 125, 128
zoographi, Concha longa 383
Zygochlamys patagonica 252, 260, 262

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VOL. 53, NO. 2 MALACOLOGIA 2011

CONTENTS

FULL PAPERS

Frank Köhler
Australocosmica, a new genus of land snails from the Kimberley, Western Australia (Eupulmonata, Camaenidae) 199

Yi Yuan, Huifen Dong, Xingjian Xu, Guiling Li, Fenghua Wei, Yunbing Zhao, Zuwu Tu, Min Liu, Mumin Cao, Hui He, Li Tang, Hong Zhu & Hongping Fan
Evaluation of a new molluscicide for counteracting the intermediate snail host of *Schistosoma japonicum* 217

Maria I. Gomez, Ellen E. Strong & Matthias Glaubrecht
Redescription and anatomy of the viviparous freshwater gastropod *Hemisinus lineolatus* (W. Wood, 1828) from Jamaica (Cerithioidea, Thiaridae) 229

Diego G. Zelaya, Laura Schejter & Cristián Ituarte
Neactaeonina argentina, new species, and family placement of the genus *Neactaeonina* Thiele, 1912 (Mollusca: Gastropoda) 251

Robert T. Dillon, Jr.
Robust shell phenotype is a local response to stream size in the genus *Pleurocera* (Rafinesque, 1818) 265

Folco Giusti, Viviana Fiorentino, Andrea Benocci & Giuseppe Manganelli
A survey of vitrinid land snails (Gastropoda: Pulmonata: Limacoidea). . . . 279

RESEARCH NOTES

Oliver Müller & Bruno Baur
Survival of the invasive clam *Corbicula fluminea* (Müller) in response to winter water temperature 367

Marina Güller & Diego G. Zelaya
On the generic allocation of *"Aligena" pisum* Dall, 1908 373

HISTORICAL NOTE

Arturo Valledor de Lozoya & Rafael Araujo
How the naiad was drawn: a pre-Linnean iconography of freshwater mussels 381

INDEX 403

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